Naval Health Research Center Detachment (Toxicology)

COMMENTARY AND SCIENTIFIC REVIEW OF STUDIES ON CARDIAC FUNCTION AND CARDIAC SENSITIZATION MODELS

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Interim Report
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Executive Summary

Problem:

During the 1980's and into the 1990's, international agreements were developed that led to the phase out of the chlorofluorocarbons (CFC) because of the demonstrated effect these compounds have on depleting the protective ozone layer in the upper atmosphere. Because of the elimination of these compounds, the chemical industry began to introduce replacement compounds that have little or no effect on the ozone layer. These compounds, the hydrochlorofluorocarbons (HCFC) and hydrofluorocarbons (HFC), were introduced as replacements for the CFC in a variety of applications including refrigeration, aerosol propellants and fire extinguishing agents. In the last 15 years, several toxicological testing and research programs were initiated to examine the potential adverse health effects of these new alternatives. For the class of fluorocarbons, cardiac sensitization[1] remains a potentially serious health outcome of overexposure to these compounds. Although routine toxicological testing of these compounds normally includes an evaluation of this endpoint, it would be advantageous to the military use of these compounds if methods were developed that would allow prediction of cardiac sensitization before the onset of a fatal arrhythmia.

Military Relevance:

Replacement of CFC fire suppressants and refrigerants in existing military systems with suitable alternatives has been directed in order to comply with international agreements that ban the production and limit the use CFCs determined to be ozone depleting substances (ODS). To address this requirement the Department of Defense has funded programs such as the Strategic Environmental Research and Development Program and the Next Generation Fire Suppression Program to find suitable replacements for ODS currently in use. Selection of suitable replacements is a difficult and complex process which has proven costly and time consuming. The complexity of the problem is illustrated by the need to evaluate several possible replacement candidates for specific applications in terms of cost and impact on operational readiness, safety, and survivability factors associated with weapons systems. For example, iodotrifluoromethane (CF_3I) has been proposed as a potential replacement for bromotrifluoromethane (commonly referred Halon 1301) as the inerting agent in wing fuel tanks on the F-16 fighter aircraft. US Air Force, Foreign Military Sales and European Participating Government costs for this proposed retrofit of the F-16 has been estimated to be in excess of $95 million. The Air Force spent 6 years and over $6 million evaluating CF_3I as a potential replacement for Halon 1301 and decided against its use on the basis of material compatibility, low temperature performance, toxicity and atmospheric chemistry issues. Subsequently, an independent review panel (IRP) of scientists, convened at the request of the Director of Defense Research and Engineering, confirmed this decision. Among the factors leading to the IRP decision was a paucity of information regarding comparative cardiac sensitization potential of these two compounds, and possible need for a more rigorous testing approach toward assessing the cardiac sensitization potential of candidate

[1] Cardiac sensitization is defined as an increased sensitivity of the heart to circulating blood levels of adrenaline (epinephrine) in the presence of a sensitizing agent, e.g., halogenated hydrocarbons, fluorocarbons. Cardiac sensitization results in the potentially life-threatening arrhythmia.

materials that would provide quantitative estimates of cardiotoxicity risk in realistic exposure scenarios.

Objective:

A research program in conjunction with Ohio State University was undertaken to identify in experimental animals those cardiac parameters, e.g., heart rate, cardiac stroke volume, etc., that could be used as predictors of cardiac sensitization. Drugs (cardiac glycosides) known to affect cardiac function were used as model compounds to cause changes in cardiac function that would then be measured using probes implanted in the region of the heart. From these data, mathematical descriptions (logistic models) were constructed to allow for prediction of the onset of arrhythmia prior to initiation of a cardiac sensitization outcome. This research resulted in the creation of three manuscripts to be published in peer-reviewed journals. All three publications, however, were rejected multiple times for publication in a peer-reviewed journal. The objectives of this report, therefore, were to ensure that the data in the manuscripts was maintained and to provide an expert commentary on ways to improve these papers through editorial changes and initiation of other research objectives.

Results:

The results provided in the three manuscripts represented an appropriate beginning to a basic understanding of cardiac sensitization and the prediction of this outcome based on the occurrence of arrhythmia. Data were presented in the papers showing that cardiac function can be accurately measured with the insertion of probes into the region of the heart. In addition, cardiac changes were assessed in the presence of cardiac glycosides. All of these data were then incorporated into a logistic model that appeared to be predictive of a pre-arrhythmogenic change. Subsequently, the data were presented in three papers. The manuscripts, however, will need extensive revisions to convey the scientific message of prediction of cardiac sensitization. Furthermore, on the part of the authors, there appeared to be some misinterpretation of cardiac sensitization. The majority of the revisions required in these papers are editorial in nature, although a restructuring of the papers through data transformation, combining of data presented in tables and figures and consolidation of two papers into one manuscript would greatly add to the clarity of the research presented. With additional support the deficiencies in the papers can be overcome.

The logistic model presented in one manuscript appeared to be predictive of the arrhythmia based on measurement of heart rate. Therefore, the usefulness of this mathematical description would appear to be useful for prediction of cardiac sensitization. Additional research would be needed to further validate the model and to demonstrate that the model is predictive of the biological response. These additional data can be generated with some investment, and the outcome would greatly enhance the protection of military personnel using this class of fluorocarbon compounds.

Conclusions:

The purpose of this report was to review the outcome of research generated for the prediction of cardiac sensitization based on measuring cardiac function in the presence and absence of drugs known to affect cardiac function. In addition, guidance on editorial changes and additional testable research objectives were to be provided on three manuscripts proposed for publication in a peer-reviewed journal. Based on the review of the research and papers, the following conclusions are made:

1. The original research objectives included in all three manuscripts are valid, i.e., the prediction of cardiac sensitization based on measuring various parameters of cardiac function.
2. With some investment of time and other resources, the three proposed manuscripts could be improved to provide publications that could potentially be published in a peer-reviewed journal. Many of the changes required for these papers are editorial in nature although data transformation and consolidation of some of the tables and figures would be necessary.
3. The mathematical description presented in two of the papers appears to be appropriate for its intended purpose, i.e., prediction of cardiac sensitization. Additional testable research objectives would be necessary to further validate the model as well as provide assurances that it is predictive of the biological consequences of exposure to fluorocarbons.

Introduction

In the early 1970's, concerns were raised about the possible depletion of the ozone layer by chlorofluorocarbons (CFC) in the landmark paper by Molina and Rowland (1974). In the years that followed, numerous research programs were initiated further examining the role of the CFC and other halogenated hydrocarbons in the depletion of ozone in the upper atmosphere. Because of the concern over ozone depletion, international agreements regarding the phase out of the CFC were developed and culminated in the Montreal Protocol and its subsequent amendments.

In addition to the research on the mechanism of ozone depletion, several major chemical companies began an intensive search for alternatives to the CFC. This research led to the development of hydrochlorofluorocarbons (HCFC) and the hydrofluorocarbons (HFC). These replacement compounds were commercially developed for various applications including refrigeration and as aerosol propellants and fire extinguishing agents. Testing for the safety of these chemicals became a priority, and most of the chemical companies participated in industry sponsored testing programs that received international attention. One such industry sponsored consortium was the Program for Fluorocarbon Toxicity Testing (PAFT). In this testing consortium, several of the new CFC alternatives were examined for toxicity, and included short and long-term testing but also included specialized testing such as cardiac sensitization. Cardiac sensitization has represented a potential adverse human health effect for many decades, and methods to detect this endpoint in experimental animals have varied over the last 20 years. Regardless, the methodology (see below) results in the generation of a no-observable-adverse-effect level (NOAEL), and a lowest-adverse-effect level (LOAEL). The LOAEL is defined as the lowest concentration tested that result in the occurrence of a response. In the case of cardiac sensitization, a cardiac sensitization response in one experimental animal would represent the LOAEL. A more detailed description of cardiac sensitization is presented below.

The use of the CFC alternative in certain applications, e.g., fire extinguishing, results in a greater potential for human exposure. Other than controlling the potential for exposure, it would be useful to predict the onset of cardiac sensitization through greater understanding of the events leading to this endpoint. By understanding the early arrhythmogenic effects of the fluorocarbons, greater assurance of the protection of military personnel is achieved. Research on the pre-arrhythmogenic effects of drugs know to affect cardiac function was undertaken. In that research, probes were inserted into the region of the heart, and various parameters measured, e.g., heart rate, stroke volume and pressure, etc. From these data, a mathematical description of these parameters was constructed that would allow the prediction of these pre-arrhythmogenic events. All of this research was compiled into three separate manuscripts for publication in a peer-reviewed journal. These papers, however, were not accepted for a number of reasons.

ENVIRON was asked to review and potentially re-write three proposed manuscripts for Geo-Centers, Inc. The draft papers submitted for review were:

- Nakayama, T., Powers, J., Herderick, E.E., Briggs, G.B., Still, K.R., Smith, E.A. and Hamlin, R.L. Effect of autonomic tone on the arrhythmic dose of ouabain on various physiological parameters preceding ventricular ectopy.

- Herderick, E.E., Powers, J. Nakayama, T., Briggs, G.B., Still, K.R., Hamlin, R.L. and Smith, E.A. A logistic regression model to predict onset of cardiac arrhythmia.

- Smith, E.A., Nakayama, T., Herderick, E.E., Powers, J., Briggs, G.B., Still, K.R. and Hamlin, R.L. A new perspective for identifying potential cardiac sensitizers.

An initial, brief review of these papers suggested that they could be re-written for publication with some effort, although the latter two papers could be usefully combined into one manuscript. On a more thorough review, however, it was quickly realized that a more extensive data transformation and analysis would be minimally required before any attempt at re-writing could be undertaken. Furthermore, insufficient data were presented in the latter two papers to ensure that the logistic model was predictive of the biology of arrhythmia. Therefore, additional research might be required to demonstrate validity of this model. Once these facets of the research program are completed, then additional research objectives could focus on detection and prediction of cardiac sensitization.

A major emphasis of these papers was to identify certain cardiac parameters that could be used to predict cardiac sensitization. It became clear during the review of these papers that the focus should be directed towards a better understanding and measurement of cardiac function. In addition, a review of these papers suggested that there was no clear understanding of the phenomenon of cardiac sensitization. Therefore, for the purposes of this report, a brief review of cardiac sensitization seems appropriate.

Cardiac Sensitization – Development and Methods

In the early 1900's, Levy and Lewis (1911) reported that cats lightly anesthetized with chloroform were unexpectedly sensitive to injected epinephrine. In these studies, the investigators administered chloroform at 0.5% or 2% in air followed by a bolus intravenous injection of epinephrine (up to 65 μg total dose). The authors described the electrocardiographic (ECG) pattern as "heterogenetic", short pauses in heart rate followed by tachycardia. Continued administration of chloroform ultimately resulted in ventricular fibrillation. Levy followed up on this initial work and reported variations in cardiac sensitivity that was dependent on the duration and degree of anesthesia (Levy, 1913). He found that cats under light anesthesia were more susceptible to the cardiotoxic effects of chloroform compared to deeper surgical anesthesia. This response appeared to be related to a decrease in central nervous impulses to the heart, although Levy had not characterized this possible mechanism. In a review of the literature, Levy found a number of cases where humans had been overcome by chloroform. In these cases, he reported that medical treatment consisted of injecting epinephrine (to stimulate the cardiovascular system) but, in many cases, the patient subsequently died after exhibiting tachycardia followed by ventricular fibrillation. This increased sensitivity of the heart to epinephrine brought about by exposure to a specific organic chemical was referred to as cardiac sensitization.

Subsequent to the work of Levy, many studies were conducted to characterize the ability of general anesthetic agents to produce cardiac arrhythmia and sensitivity of the myocardium to epinephrine. Examples include studies on cyclopropane, ether, halothane and trichloroethylene (Meek, et al., 1937; Orth, 1939; Waters, et al., 1943; Geiger, 1943; Smith, et al., 1962; Forbes,

1966). Additional studies were carried out on potential anesthetic agents or similar compounds including cyclic and acyclic hydrocarbons, unsaturated hydrocarbons and certain fluorinated hydrocarbons (Carr, et al., 1949; Krantz, et al., 1948; Burgison et al., 1955).

Based on these and other studies, the potential hazard associated with administering hydrocarbon anesthetic agents followed by epinephrine became clearly recognized. It was not until the 1960's when the increased use of chlorofluorocarbons (CFC) as aerosol propellants and their deliberate misuse resulted in numerous sudden deaths that cardiac sensitization became once again a phenomenon needing further investigation.

The chlorofluorocarbons were first developed in the 1930's and eventually found use in refrigeration, replacing the more toxic ammonia and sulfur dioxide. Early research and testing with these chemicals revealed that, as a class, these compounds were quite stable under use conditions and were very low in toxicity. In the 1940's and 1950's, the CFCs were found to have certain physical and chemical properties that allowed their use in other specialized applications. CFCs were then used as solvents, foam-blowing agents, fire extinguishing agents and as aerosol propellants. Much of the heightened concern during the 1960's was the abusive use of CFC propellants to achieve a light anesthesia. In this application, the user attempted to get "high" (stage I anesthesia) similar to that described for illicit drug use. However, there were numerous fatalities from "sniffing"[2] of the propellants (Reinhardt, et al., 1971). The deliberate, abusive "sniffing" of aerosol products and propellants to achieve a state of euphoria began in the early to mid-1960's and peaked in the late 1960's and early 1970's (Bass, 1970). Deaths from aerosol "sniffing" were always sudden, occurred during or shortly after inhalation of high concentrations of the aerosols, and were generally accompanied by physical exertion or some type of stress (for example, extreme excitation). The mechanism for most of these deaths was thought to be due to cardiac sensitization occurring from the inhalation of high concentrations of aerosol propellants, coupled with corresponding high blood levels of endogenous adrenaline, resulting in the sudden onset of ventricular fibrillation. Autopsy of those that died from this abuse generally do not present any unusual findings. No anatomical changes are observed in the heart, brain or other organs. The diagnosis of cardiac sensitization is usually based on circumstantial evidence at the scene, i.e. position of the body, empty aerosol cans, and a lack of autopsy findings that might otherwise be responsible for the death. Because of the increased illicit use of the CFC propellants, additional research was undertaken to describe this phenomenon in more detail.

Several groups undertook a search for an animal model and the development of a methodology to predict cardiac sensitization potential. The most active groups during the period of the late 1960's and into the 1970's were Reinhardt and colleagues at DuPont' Haskell Laboratory and Clark and Tinston at ICI's Central Toxicology Laboratory. The research conducted by both groups centered primarily on selection of an appropriate animal model and simulating increases in blood adrenaline levels by intravenous injection of appropriate doses of epinephrine. The various methods for assessing cardiac sensitization and the interpretation of the results in risk assessment modalities have been recently reviewed (Brock, et al., 2003).

The prediction for the potential to induce cardiac sensitization in humans has been raised as means to control exposures to sensitizing agents. Although it is clear that structure-activity determinations will allow an assessment of the compound to be inherently a sensitizing agent, it

[2] Aerosol "sniffing" is a misnomer. "Sniffing" is the deliberate, deep inhalation of highly concentrated vapors.

is the potency of the compound that becomes important when exposures occur in different applications. In the current sensitization methods, the potency of a compound can be determined, but only in relation to a previously known standard, generally fluorocarbon (FC) 11 or Halon 1301. Another feature of importance in predicting cardiac sensitization is the time to onset of cardiac arrhythmia with exposure since this may lead to a fatal outcome of exposure. Clearly, there will be a relationship between blood levels of the agent (internal dose) and adrenaline, and the duration of exposure.

Blood Pharmacokinetics and Modeling

Results from several studies have revealed the relationship between sensitizing blood concentration of the compound and the duration of exposure before giving the epinephrine challenge. In these studies, it became clear that the concentration of the compound at a peak time was the most important factor. Studies of dogs exposed to Halon 1211 (Beck et al., 1973) receiving an epinephrine challenge at various times during the exposure produced similar venous blood concentrations associated with cardiac sensitization. Exposures for 1, 2, or 5 min at concentrations of 8%, 5%, or 2% resulted in venous concentrations of 21, 23, and 24 μg/mL, respectively. Furthermore, Beck et al. (1973) demonstrated that it did not matter whether the peak concentration was reached quickly by a higher exposure concentration or more gradually by a lower exposure concentration. These results are further supported by studies conducted earlier at Haskell Laboratory. Exposure of dogs to CFC-12 for 5 min (Reinhardt et al., 1971) resulted in a cardiac sensitization response at 5% but not at 2.5%. A longer exposure durations of 30 or 60 min to 2.5% still resulted in no cardiac response. However, while brief exposures with epinephrine challenge at 0.5 minutes produced no response at 7.0%, 3 of 7 dogs did respond at a concentration of 13.5%.

Like most short-chain halocarbons and hydrocarbons, CFC-12 is only slightly soluble in blood, is readily absorbed from the lungs into the bloodstream where it rapidly equilibrates as a function of the blood:air partition coefficient, and essentially reaches steady-state within minutes (Azar et al.1973). Upon cessation of exposure, blood concentrations of CFC-12 rapidly decrease. This same pattern of blood uptake and elimination has been shown for CFC-11 (Azar et al. 1973), CFCs 113, 114 and 115 (Trochimowicz et al. 1974), and Halon 1301 (Mullin et al. 1979). The increase in blood concentrations observed for these fluorocarbons clearly indicate a concentration times time (Cxt) relationship with steady-state occurring within about five minutes. Longer duration exposures do not significantly change the plateau blood concentration of most fluorocarbons. Furthermore, it is apparent that the peak blood concentration of a fluorocarbon prior to epinephrine challenge, given iv at 5 min after initiation of agent exposure, is directly related to the induction of cardiac sensitization. Therefore, the risk of exposure associated with this class of compounds (halocarbons and some hydrocarbons) is a function of steady-state blood concentration which is both time and chemical dependent.

It would follow, therefore, that one might be able to predict, based on various pharmacokinetic parameters, the blood concentration of an agent and duration of exposure needed to achieve a critical blood concentration. A tool that allows the investigation of these pharmacokinetic factors is the physiologically-based pharmacokinetic (PBPK) model. Such models allow one to examine the relationship between external exposure scenarios and internal

concentrations at a target tissue, i.e., blood. The model incorporates a mathematical description of the uptake, distribution, metabolism, and elimination of chemicals by the body. Modeling of airborne exposure to cardiac sensitizing agents requires that the model account for short-term (0 to 5 min) events. Vinegar et al. (1998) described such a model. This model included a respiratory-tract compartment containing a dead-space region and a pulmonary exchange area. The pulmonary exchange area contained air space, tissue, and capillary subregions. Respiratory-tract uptake was described on a breath-by-breath basis that allowed successful simulation of exhaled breath concentration during the first minute of exposure. Furthermore, Monte Carlo simulations can be included that allows for variations in a population.

The validated PBPK model can be used to assess the potential risk to cardiac sensitizing agents in a number of ways. Each cardiac sensitization method, however, depends on the determination of the critical blood concentration, typically the peak blood concentration resulting from exposure to the lowest-observable-adverse effect level (LOAEL). These data are not often available from dog studies and less frequently available for humans, the target population. The most direct way to obtain this value is to perform a pharmacokinetic study with arterial and venous samples taken at various time points during exposure. Since the exposure level of interest is the threshold (LOAEL) concentration for cardiac sensitization, the pharmacokinetic study should be performed at this exposure concentration, but without epinephrine challenge. The alternative is to use a PBPK model to simulate the blood concentration at the critical airborne exposure level, e.g., the cardiac sensitization LOAEL.

Review of Manuscripts

In fire extinguishing or solvent applications using fluorocarbons or other halogenated hydrocarbons, implementing engineering controls is certainly a better means to control potential exposures to workers in order to avoid inducing cardiac sensitization or other toxicities. It would seem reasonable, however, that in the absence of full knowledge of exposure concentrations a mathematical model could be constructed that would allow one to predict the probability of occurrence for the onset of arrhythmia for a specific chemical. In developing such a model, an understanding of the possible mechanism of action of cardiac sensitization should be investigated to ensure that the appropriate endpoints of arrhythmia are determined to construct a predictive model. Any mathematical model can be constructed. It must, however, be predictive of the biological response.

Because of substantive issues with the papers and limited funding to support additional research at this time, an approach is being taken to ensure that the data presented in these papers are immortalized. The purpose of this section of this report is, therefore, to provide a scientific and editorial review of each paper, and make recommendations for data analysis. Secondly, on a broader scale, additional research objectives will be suggested that should assist in developing new data that would allow the logistic model to be tested for its ability to predict the onset of cardiac arrhythmia. The papers reviewed are enclosed as Appendix I.

Nakayama, T., Powers, J., Herderick, E.E., Briggs, G.B., Still, K.R., Smith, E.A. and Hamlin, R.L. Effect of autonomic tone on the arrhythmic dose of ouabain on various physiological parameters preceding ventricular ectopy.

Introduction:

This manuscript needs to focus on the methodology for collecting cardiovascular function data. The secondary objective could remain, i.e., how parasympathetic and adrenergic blockade affects the values of cardiovascular function. The ultimate research objective should be explained in the introduction that the investigations are designed to assist in the development of predictive models of arrhythmia with exposure to fluorocarbons and other halogenated hydrocarbons. Indeed, reference to the other manuscript(s) could be appropriate.

In the review of Reynolds (1984), the mechanism of cardiac sensitization is postulated as a shift in electrical conductance from the sinoatrial (SA) node to the atrioventricular (AV) node resulting in arrhythmia. The author is quick, and correctly, to point out that this may be one of several interdependent mechanisms. Citing this paper and related references will help set the tone of the manuscript as an initial investigation in cardiac function with ouabain used as a model compound for eliciting changes in cardiac function. It would be critical not to refer to ouabain as a cardiac sensitizer since this has not been shown for this drug.

Finally, there is an excessive use of references. A balance needs to be achieved between citing too many references and citing only those references that support a conclusion.

Methods:

Additional detail is needed in this section of the manuscript. At minimum, information on the age and weights of the dogs used for the study needs to be included as well as how dogs were assigned to experimental groups, e.g., random assignment. The paper of Lang, et al. (1992) should be cited as a basis for the use of chlorlase and morphine anesthesia in this study since certain anesthetic agents will affect cardiovascular function.

A major problem with this manuscript is the large variation in measured parameters among control animals such that statistical comparisons with treated groups become problematic. Although this may be difficult to overcome, expert statistical consultation should be sought to examine whether data transformation would be appropriate. For example, arcsin transformations are done to statistically "smooth" certain data, e.g., cytogenetic data. A similar transformation could be considered for these cardiovascular data. Until this problem is overcome, it may difficult to have this paper accepted into a peer reviewed journal.

Including atropine and atenolol in this investigation is a unique addition and, as pointed out in the original manuscript, could have clinical significance. Cardiovascular data from the combination of the two drugs needs to be examined for statistical comparisons with transformed control data undertaken. It should be noted that one reviewer of this paper mentioned that the half-life ($t_{1/2}$) of atenolol was 1 hr. A review of literature, however, revealed that the $t_{½}$ of this drug in humans was 3-5 hrs with peak plasma level occurring about 2 hrs after an oral dose (Hardman, et al., 2001). The half-life in dogs would not be expected to be radically different, although this would need to be confirmed.

Results:

In its present form, Table 1 of original manuscript is difficult to follow given the multiple comparisons, the number of parameters and the means of data presentation, e.g., changes in parameters from a baseline measure. Once the data are transformed and statistical comparisons are made, consideration should be given to limiting the number of parameters presented based on statistical significance and, more importantly, those deemed to be of biological consequence. For example, changes in heart rate were suggested by Reynolds (1984) to be a sensitive indicator of cardiac sensitization, a parameter that the authors measured. Therefore, greater effort should be undertaken to identify the sensitive measure in this research initiative.

Discussion:

The focus of this section of the paper should reflect the results after transformation (if possible). Furthermore, it would be appropriate to discuss the findings in relation to potential mechanisms of cardiac sensitization knowing that the ultimate research objective is the prediction of arrhythmogenic effects of fluorocarbons. As noted above, Reynolds (1984) provides useful a literature review on this topic. Finally, the clinical significance of this study in relation to the use of atropine and atenolol would be useful.

Overall, this paper has the better possibility of getting published of the three manuscripts submitted for review. The limitation to this paper is the need to statistically re-examine the data. Once this is done and the manuscript re-written to reflect changes noted above, it might be acceptable. Currently, no additional experiments would be needed for this paper.

Herderick, E.E., Powers, J. Nakayama, T., Briggs, G.B., Still, K.R., Hamlin, R.L. and Smith, E.A. A logistic regression model to predict onset of cardiac arrhythmia.

Smith, E.A., Nakayama, T., Herderick, E.E., Powers, J., Briggs, G.B., Still, K.R. and Hamlin, R.L. A new perspective for identifying potential cardiac sensitizers

Because of the similarity of methods and data in these two papers, they will be reviewed together. Indeed, once the faults of these papers are overcome, the two papers should be combined into one manuscript. The comments that follow should be considered in the context of re-writing these papers as one manuscript.

The focus of the manuscript needs to change from prediction of cardiac sensitization to one of predicting arrhythmia. In the experiments that were undertaken, the investigators examined cardiac arrhythmia in the presence of ouabain, and assume in the draft manuscript that arrhythmia is a measure of cardiac sensitization. This is an incorrect assumption. Ouabain has not been shown to be a cardiac sensitizer. In addition, no attempt was made to administer epinephrine or stimulate the release of adrenaline.

Because of the issues associated with these manuscripts, editorial comments at this point would not be valuable. Therefore, this part of the review will concentrate on experimental

objectives to improve the quality of the data to potentially demonstrate the usefulness of this model.

The logistic model presented (Herderick, et al.) appears to be predictive based on some parameters, e.g., reduced QT interval and increased dP/Tmax, with varied doses of ouabain. What is uncertain is whether this is a unique finding to ouabain or whether this can be extended to other cardiac glycosides. An issue for any mathematical model is whether it reflects the biology. Insufficient data are currently available in the papers to validate the model. Therefore, initial research objectives should be aimed at ensuring there is a relationship between cardiac arrhythmia and the potential to describe the arrhythmia with a mathematical model. Also, since the overall objective of this research plan is to predict arrhythmia related to cardiac sensitization, the effects on this model with known cardiac sensitizers will need to be examined. Several research objectives, therefore, will need to be considered.

1. Examine the role of a diverse group of compounds known to affect cardiac function and collect data as described in the paper drafted by Nakayama, et al. If the model can be used to predict arrhythmia, then the parameters that changed in the presence of ouabain should be consistently observed with other compounds. Also, epinephrine must be included in these experiments since this material is the basis for inducing cardiac sensitization, and include a dose-titration experiment to determine an optimal dose for inducing changes in cardiovascular function[3]. Given the extensive literature on epinephrine and cardiac function, the compound could be used as a test for the sensitivity of the research model. Because of the inter-relationship of the parameters that have been measured in these papers, the investigators will need to follow the development of arrhythmia, changes with time, rather than the ultimate occurrence of arrhythmia. As noted above, Reynolds (1984) provides good mechanistic arguments on this point. Clearly, the sensitivity and the reproducibility of the parameters will determine the validity of the model description.

2. Examine compounds not suspected of inducing changes in cardiovascular function. For example, it would be useful to examine fluorocarbons or other halogenated hydrocarbons in the absence of epinephrine. These compounds generally do not affect cardiac function in the absence of epinephrine. These experiments should be conducted without co-administering epinephrine and would, therefore, serve as a baseline measurement for experiments to follow. These studies will need to be conducted at concentrations expected to induce cardiac sensitization. Caution will need to be exercised in these experiments to ensure that the experimental animal (dog) is not anesthetized or "stressed", the latter situation having the potential to release endogenous adrenaline and thereby inducing cardiac sensitization and causing changes in other cardiac parameters.

[3] Because of the procedures used by Nakayama to collect data, i.e., probes being placed in blood vessels of the heart, the dose of epinephrine used in typical cardiac sensitization protocols may be too high for these experiments. Therefore, dose titration experiment will need to be conducted. Furthermore, a baseline of cardiovascular changes in the presence of epinephrine needs to be determined prior to conducing the final phase of the research where cardiac changes are measured in a typical cardiac sensitization protocol.

3. After completion of the above research, a series of experiments should be undertaken with the combination of epinephrine and known cardiac sensitizers. Dose-response data for the compounds will need to be developed within this research objective to ensure optimum doses of the, e.g., fluorocarbon, are used in these experiments.

Throughout this research program, the investigators will need to examine compounds administered by inhalation since this route of exposure represents the greatest risk to military personnel. Furthermore, as noted to above, the experimental animal should be the dog since this species has historically been used in cardiac sensitization studies. Finally, at each phase of the research program, the mathematical model will need to be tested against the collected data to ensure that the sensitivity and specificity of the model continues to provide predictive value.

Summary and Conclusions

There is a clear need for health scientists to develop a means to improve our ability to protect workers handling potentially toxic chemicals. Using computer assisted models to improve the technology of worker protection will ultimately lead to reduced animal use. In the research manuscripts provided by Geo-Centers, the goal of the research program was to develop a logistic model to allow prediction for the potential of cardiac sensitization in military personnel using fluorocarbons or other halogenated hydrocarbons. Although the model appeared to be predictive of, at least, heart rate, there was no basis to ensure that the model was related to the biology of cardiac sensitization or that the model was sensitive and reproducible. This review attempted to provide scientific guidance on changing the focus of the papers as well as describe other research objectives that would allow further development and validation of the model. The basis for those research objectives was to develop data consistent with the phenomenon of cardiac sensitization and further test the model to ensure the prediction of the model. Finally, as the data are developed from this research, the model would be continually tested for specificity. In the paper by Nakayama, in spite of the methodological and data presentation issues, the experimental design and data collection methods were clearly valid and useful. With some effort on editorial changes and data transformation, this paper could be easily re-written and submitted for publication.

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EFFECT OF ALTERED AUTONOMIC TONE ON THE ARRHYTHMIC DOSE OF OUABAIN AND ON VARIOUS PHYSIOLOGICAL PARAMETERS PRECEDING VENTRICULAR ECTOPY.

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Abstract

EFFECT OF ALTERED AUTONOMIC TONE ON THE ARRHYTHMIC DOSE OF OUABAIN AND ON VARIOUS PHYSIOLOGICAL PARAMETERS PRECEDING VENTRICULAR ECTOPY.

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Although many studies of digitalis-induced ventricular ectopy have been conducted, including investigations of the mitigating effects of autonomic efferent activity, we know of no study that examines the influence of changing autonomic activity on the parameters of cardiovascular function associated with the development of ventricular ectopy. The doses of ouabain required to produce a ventricular arrhythmia, either de novo or provoked by programmed electrical stimulation, and the values of physiological parameters preceding, were studied in dogs anesthetized with morphine/chloralose, and instrumented to record important, clinically relevant parameters of cardiovascular function. Measurements were made during a control period and after graded dose of ouabain up to when ventricular ectopy developed. Dogs were studied with intact autonomic control and after parasympathetic blockade by atropine, $beta_1$ adrenergic blockade by atenolol, and complete pharmacologic denervation using both. There were no differences in baseline heart rates, except for the higher rates in dogs given atropine. The dose of ouabain (from 47 to 57 μg/kg) necessary to provoke an arrhythmia did not differ among the groups, although it tended to be lower in groups receiving atropine. Thirty-seven percent of the dogs developed arrhythmias with programmed electrical stimulation, while the remainder of the dogs developed arrhythmias without provocation. Measures of myocardial contractility and systemic arterial smooth muscle tone all increased and QTc shortened, just before ventricular ectopy. The only difference in parameters before ectopy was in QTc, which shortened less with atropine.

Key Words: Cardiac Arrhythmia; Ventricular Ectopy; Cardiovascular Function Parameters; Ouabain; Beagle Dog.

INTRODUCTION

Digitalis, when used clinically may produce cardiotoxicity (Hardman *et al.* 1996, Rosen *et al.* 1975, Mahdyoon *et al.* 1990), and purposeful intoxication with digitalis has been used often to study arrhythmogenesis (Rosen *et al.* 1975, Cagin *et al.*1974, Lathers and Roberts 1980). It is well-known that digitalis interacts-both therapeutically and toxicologically-with the autonomic nervous system (Daggett and Weidfeldt 1965, Erlij and Mendez 1964, Gillis 1969, Gillis and Quest 1979, Hewett and Rosen 1984, Lathers *et al.*1974, Lathers and Schraeder 1987, Levitt *et al.* 1976, Levitt *et al.* 1970, Rosen 1981, Somberg 1985, Watanabe 1985). However we know of no studies which discuss how parameters of cardiovascular function, that precede development of ventricular ectopy, are altered by changing autonomic activity. In particular, no study has characterized, in a single model, parameters of ventricular and vascular function. Such information could prove valuable to the clinician that uses digitalis glycosides concomitantly with compounds (e.g. parasympatholytics, beta-adrenergic blockers) known to affect autonomic balance. This study would also assist clinicians, or toxicologists, who seek physiological parameters that may precede ventricular ectopy produced by toxic doses of digitalis glycosides. As such, the purposes of this study are: (1) to determine if the toxic dose of ouabain changes with parasympathetic and/or beta adrenergic blockade, and (2) to determine the effects of changing autonomic tone on values, or change in values, of specific parameters of cardiovascular function that may precede the development of ventricular ectopy.

METHODS

Baseline treatment regime: Forty-eight, young-mature, healthy, beagle dogs were anesthetized with morphine (0.25 mg/kg given im) and chloralose (100 mg/mg given iv). Anesthesia was sustained with chloralose (25 mg/kg/hour). Dogs were intubated and ventilated with room air at a frequency of 15 breaths per minute and a tidal volume of 15 ml/kg. This ensured that systemic arterial Pa02 was always greater than 85 mmHg and $PaCO_2$ was always between 35 and 45 mmHg. Catheter-tip micromanometers were placed in the left ventricle and ascending aorta, while fluid-filled catheters were placed in the right atrium and pulmonary trunk. Electrodes forming ECG leads I, aVF, V10 and V3 were attached. A bipolar pacing catheter was placed in the right ventricle.

Basline parameters: Pressures (P) were recorded from the left ventricle (LV), ascending aorta (Ao), right atrium (RA) and pulmonary trunk (PA). Cardiac output (CO) was measured in triplicate by thermodilution after injecting room temperature saline into the RA and sampling temperature in the PA. Using the LVP, values for end-diastolic pressure (EDP), dP/dtmax[1], tau[2] and dP/dt/P extrapolated to the P0 intercept (vmax)[3], were measured or calculated (Little and Cheng 1991, Peterson *et al.* 1974, Weiss *et al.* 1976). Systemic vascular resistance (SVR) was estimated as (PAo-PRA)/CO and pulmonary vascular resistance (PVR) was estimated as PPA/CO. Stroke volume (SV) was calculated by dividing CO by heart rate (HR). Approximations of vascular impedance (Z)[4] were made for the Ao and PA by dividing pulsatile pressure by SV.

Repetitive ventricular responses (RVR)[5] were sought by programmed electrical stimulation (PES). This was accomplished using 8 conditioning stimuli (S1)[6] with an interval of 250 ms, followed by a 9th (S2) approximately 15 ms after the effective refractory of the last of the conditioning stimuli, and a 10th, (S3), which was approximately 15 ms after the 9th. An RVR was considered 3 unpaced ventricular extrasystoles after the 10th stimulus. The effective refractory period of the right ventricular endocardium was taken as the longest coupling interval between the last conditioning stimulus and the 9th conducted stimulus. QT was corrected (QTc) for heart rate (HR) using the cube root method ($QTc=QT/(RR)^{1/3}$). RR is the interbeat interval preceding the QT that was measured.

Ectopy onset: Measurements of all parameters were made during a control period and during the final minute of a 15-minute period during escalating doses of ouabain. Ouabain is characterized as a rapid-acting digitalis glycoside; thus all parameters of cardiovascular function monitored had changed maximally within 15 minutes. Since the half-life of ouabain is greater than 24 hours and the experiments terminated within 1.5 hours of dosing, the effects of ouabain were truly cumulative. Experiments were concluded when ventricular ectopy occurred either spontaneously or was provoked by PES.

Dosage schedule: Twelve dogs were allocated to receive (1) no autonomic blockade, (2) a muscarinic, parasympatholytic dose of atropine (0.04 mg/kg given iv 10 minutes before the study), (3) a $beta_1$ adrenergic blocking dose of atenolol (50 mg/kg given po the night before and 2 hours before the experiment), or (4) both atropine and

atenolol. Control measurements were obtained, and then each dog was given intravenously 50 μg/kg of ouabain, then 15 μg/kg additional ouabain every 15 minutes.

Data recording: All recordings were made on a Biopac MP100 and data analysis was performed with Microsoft Excel.

Data analysis: Values of all parameters for all 4 groups were compared between baseline (before ouabain) and the last recording prior to the occurrence of ventricular ectopy. The total dose of ouabain required to elicit ventricular ectopy was recorded. The absolute difference and the percent change were calculated as the baseline value minus the pre-arrhythmic value and the absolute difference divided by the baseline value, respectively. Differences in means were sought by ANOVA with repeated measures design; a significant F-statistic prompted comparison of specific means by Scheffe's post-hoc analysis (requiring a $p < 0.05$ for significance).

RESULTS

Table I shows means and standard errors of the means for all parameters for all groups during the baseline recording and during the final recording prior to the development of ectopy (pre-ectopy). Differences and percent changes are shown; a negative sign preceding either the change or percent change in any parameter indicates that the value preceding ectopy was greater than the baseline value. P-values refer to differences between baseline and pre-ectopy for each group. Small letters, after each value, refer to parameters that did not differ significantly when comparing baseline values across the groups, while capital letters refer to parameters that did not differ significantly when comparing pre-ectopy values across the groups.

The dose of ouabain required to produce ectopy was consistent across the groups. However, it appears that less, though insignificantly less, ouabain was necessary to produce ventricular ectopy for dogs receiving atropine (52 μg/kg) or both atropine and atenolol (47 μg/kg) than for dogs receiving no autonomic blockade (56 μg/kg) or just atenolol (57 μg/kg). The development of arrhythmia in response to PES varied among the 4 groups of 12 dogs each. Ventricular ectopy produced by PES was produced in 5 out of 12 control dogs, in 6 out of 12 dogs receiving atenolol,

in 3 out of 12 dogs receiving atropine, and in 4 out of 12 dogs receiving both atenolol and atropine. All others developed arrhythmias without provocation. Chi-square analysis determined that there were no differences in the percentages of dogs, among the groups, developing arrhythmias by PES. Dogs receiving atropine, with or without atenolol, had a tendency to develop their arrhythmias de novo rather than by PES.

There were no differences in baseline HR's among groups of dogs except in dogs given atropine whose HR's were significantly higher (p=000). There was a significant decrease in HR preceding ectopy for controls (p=0.031) and for dogs with beta blockade (p=0.003) but not in either group receiving atropine. In fact, HR tended to increase, although insignificantly, in those groups receiving atropine.

As expected, the vagolytic action of atropine accelerated HR and decreased LEVEDP, PQ, and tau during baseline. Following atenolol and the reduction in HR, tau increased. dP/dtmax, vmax, PAo, SVR, and ZAo all increased, while QTc decreased, between baseline and pre-ectopy for all groups. All parameters that increased between baseline and pre-ectopy increased more dramatically in dogs with both parasympathetic and sympathetic blockade. QTc decreased for all groups, but decreased less in the dogs receiving atropine. See Figure 1. There were no significant differences in QRS duration, PVR, ZPA, or PRA between baseline and pre-ectopy in any of the groups.

DISCUSSION

This study was designed to determine the effects of various autonomic states on the dose of ouabain required to provoke ventricular ectopy, and to determine if various measures of physiological function, which may precede ectopy, were influenced by the autonomic state. We showed that: (1) altering autonomic effects on the heart did not change the dose of ouabain required to provoke ventricular ectopy, (2) altering autonomic efferent activity did not change the ability to provoke arrhythmia by programmed electrical stimulation, (3) measures of ventricular systolic function increased in response to ouabain, and that they increased to a greater extent in dogs with both sympathetic and parasympathetic blockade, and (4) QTc decreased in response to ouabain.

The digitalis glycoside, ouabain, was used because of its rapid onset and short time to peak effects. All glycosides are considered to be essentially identical with respect to therapeutic index or mechanism of action, therefore we believe that the observations in this study on ouabain may be extrapolated to commonly used glycosides (e.g. digoxin, digitoxin)(Dutta *et al.* 1977, Dutta *et al.* 1968). Although some glycosides fail to cross the blood brain barrier, these glycosides still produce the same spectrum of arrhythmic toxicity (Lathers and Roberts 1980). This supports the contention that digitalis glycosides produce part of their toxic effects through a portion of the central nervous system outside of the blood brain barrier, probably in the area postrema (Borison 1951, Cammermeyer 1947). Of course digitalis also produces cardiotoxicity by direct action on the heart (Rosen *et al.* 1975, Rosen and Danilo 1980, Rosen *et al.* 1973 a, Rosen *et al.* 1973 b).

While all anesthetics alter pulmonary and cardiovascular function and autonomic control, it is thought that a combination of morphine/chloralose minimizes the autonomic and biochemical effects (Rath *et al.* 1995). Animals were kept in a light plane of surgical anesthesia and were given positive pressure ventilation to sustain blood gasses within normal limits to minimize the effects of the anesthetic on pulmonary function. Regardless, extrapolation of these results to dogs that are awake or in disease states for which digitalis may be indicated must be done cautiously. For example, animals with heart failure and/or hypertrophy have (1) prolonged QT (Charlemagne *et al.* 1990, Tomita *et al.* 1994), (2) reduced parasympathetic, as well as potentially increased sympathetic, efferent activities attributed to altered baroreceptor function (Reid 1996, Wilson *et al.* 1990, Eckberg *et al.* 1971), (3) reduced myocardial contractility (Braunwald *et al.* 1976), (4) increased stiffness of the myocardium (Gaash *et al.* 1976), and (5) chronotropic incompetence from down-regulation of beta adrenergic receptors (Bristow *et al.* 1982, Bristow *et al.* 1985). This is an incomplete list of differences observed between the normal, anesthetized dog and the dog with heart failure for which digitalis might be indicated. Therefore, the parameters that changed in response to ouabain may not change, may change quantitatively differently, or may change in the opposite direction in the presence of heart failure.

In this study, cardiovascular function was monitored to assess all-important properties of the cardiovascular system (Braunwald *et al.* 1976, Cohn 1992, Katz 1992). Preload was assessed by LVEDP and PRA, while myocardial stiffness was examined with tau (Little and Cheng 1991, Peterson *et al.* 1974, Weiss *et al.* 1976). Contractility was gauged with load-dependent dP/dtmax and less load-dependent vmax. Pulmonary and systemic arterial vascular tone

was characterized by resistance and an estimate of impedance (ratio of pulsatile pressure to pulsatile flow), while overall cardiovascular function was described using cardiac output. Chronotrope and dromotrope were considered using values for heart rate and for PQ and QRS durations, respectively. Ventricular irritability was measured by routine electrocardiography and by programmed electrical stimulation. It is accepted that PES is maximally sensitive for uncovering arrhythmias produced by reentry and that ouabain produces arrhythmias from triggered activity, such as delayed after-depolarizations (Forgos 1995). In man, however, PES resulting in repetitive ventricular responses has been shown to uncover predilection for digitalis-induced arrhythmias (Somberg 1985, Vassalle *et al.* 1962). Furthermore in this study, PES was able to provoke ventricular ectopy in 18 out of 48 (37.5%) of the dogs administered ouabain. If the mechanism of digitalis-induced ventricular ectopy is triggered activity with delayed after-depolarizations, then PES must be suitable for uncovering a tendency of arrhythmia, provoked by triggered activity, at least in this model. PQ interval and QRS duration are estimates of ventricular dromotrope and since QRS duration did not change when ouabain provoked ventricular ectopy, then macro-reentry requiring either slow conduction or a long pathway must not have existed. This also supports the contention that PES may be useful for uncovering the potential for arrhythmia from triggered activity.

Although no heart rate correction exists for QT duration that is proven to be valid for the dog at all rates, the cube root function of RR interval proposed by Fredericia has been shown to result in a more constant ratio of QT:RR than the square root function proposed by Bazett (Molnar *et al.* 1996). Even better means of correction have been determined (i.e. methods which keep constant the ratio of QT to some function of RR), but these closely parallel that of Fredericia and are mathematically more complicated. All methods involving RR interval are questionable as the dog with pronounced respiratory sinus arrhythmia (Hamlin *et al.* 1966) truly has no single RR interval to be used for heart rate correction of QT.

The pretreatment of dogs with atropine, atenolol or both atropine and atenolol was used to block muscarininc parasympathetic (Eglen and Whiting 1986), $beta_1$ adrenergic (Brown *et al.* 1976, Lichten *et al.* 1977) and both muscarininc parasympathetic and $beta_1$ adrenergic activity on the heart, respectively. $Beta_2$ adrenergic and alpha-adrenergic activities were not blocked, although the dose of atenolol used may well have mildly decreased $beta_2$ activity (Frischman 1984).

It is appropriate to discuss the relationship between autonomic activity and digitalis-induced arrhythmias, and the alterations in autonomic activity which affect different physiological parameters that precede development of ventricular ectopy. Digitalis may induce ventricular arrhythmias by a direct action on the heart (Braunwald *et al.* 1976) or indirectly via the central nervous system (Watanabe 1985). Ventricular arrhythmias may be produced in Langerdorff preparations isolated completely from humoral or nervous control, confirming a direct effect on the heart (Hadju and Leonard 1959). However, digitalis also may yield ventricular arrhythmias with the application of small doses of the glycoside into various regions of the brain, with special attention to the area postrema (Lathers and Schraeder 1987). The dose of digitalis required to produce arrhythmias in a spinal preparation is much greater than in a neurally intact animal, adding further credence to the role of the central nervous system (Watanabe 1985). In contrast, ouabain may actually prevent ventricular arrhythmias provoked by hypothalamic stimulation. The mechanism for this "antiarrhythmic" effect of digitalis is multifactorial. Digitalis enhances the response of the high-pressure baroreceptors to nascent arterial pressure; this increases the intensity of afferent volleys to the medulla, and decreases sympathetic efferent volleys while increasing parasympathetic efferent volleys, both of which may be antiarrhythmic. In addition digitalis, by stimulating muscarininc vagal influences, directly inhibits the release of norepinephrine (Muscholl 1980). Furthermore, digitalis through its vagomimetic activity also directly inhibits arrhythmogenic effects of norepinephrine on Purkinje fibers (Bailey *et al.* 1979).

Considering the factors related above, it is not understood why the various autonomic blocking compounds did not affect the dose of ouabain necessary to provoke ventricular arrhythmias. In particular, why did beta blockade with atenolol not require more ouabain to provoke ventricular ectopy, and why did vagal blockade with atropine not require less ouabain to provoke ectopy? One possibility is that the autonomic effects of digitalis are so minor (compared to the direct effects) that the statistical power of this study was inadequate. This may be supported by the tendency to require less ouabain when dogs were pretreated with atropine.

It is not surprising that parameters of vascular resistance and impedance increased in response to ouabain, since digitalis glycosides are known (Gilman *et al.* 1985, Mason 1968) to activate vascular smooth muscle. Further, it is not surprising that elevation in ventricular systolic function (e.g. dP/dtmax) and in vagal activity (e.g. reduction in

heart rate), and shortening of QTc preceded ventricular ectopy. The tendency for ectopy is related to the tissue level (in the CNS as well as in the heart) of the glycoside. Both positive inotropy and negative chronotropy and the duration of QT are also related with the tissue concentration of the glycoside. Thus a certain level of positive inotropy, negative chronotropy, and abbreviation of QT must have indicated a tissue concentration of ouabain adequate to provoke ventricular ectopy. We believe that these parameters may be predictive of impending ventricular ectopy produced by digitalis. It is possible that these changes are merely coincidental with the arrhythmia, or that they represent tissue concentrations elevated enough to cause the arrhythmia. We believe that the latter possibility is more reasonable, but in any case, this study suggests that it may be feasible to predict onset of ventricular ectopy by physiological monitoring.

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Table I: Means and standard errors of the means for physiological parameters recorded during baseline and preceding ventricular ectopy for untreated (control), and for dogs pretreated with atenolol, atropine or atropine and atenolol.

	control					atenolol				
	baseline	final	delta	delta%	P value	baseline	final	delta	delta%	P value
dose		56 ± 3^{A}					$57 \pm ^{A}$			
HR	113 ± 6^{a}	94.9 ± 8.9^{A}	18.5 ± 7.5	16.1 ± 6.5	0.031	106 ± 4^{a}	85.2 ± 7.8^{A}	20.4 ± 5.2	20.7 ± 5.8	0.003
LVEDP	8.4 ± 1.0^{a}	8.0 ± 1.0^{AB}	0.4 ± 0.9	1.4 ± 9.8	ns	9.1 ± 0.9^{a}	11.3 ± 1.2^{A}	-2.2 ± 1.0	-33.6 ± 13.7	0.049
Ram	3.8 ± 0.5^{a}	4.1 ± 0.6^{A}	-0.3 ± 0.7	-23.8 ± 17.7	ns	3.8 ± 0.4^{a}	4.6 ± 0.9^{A}	-0.8 ± 0.9	-26.7 ± 21.4	ns
tau	27.4 ± 1.2^{a}	24.7 ± 1.3^{AB}	2.7 ± 0.9	9.8 ± 3.1	0.010	33.2 ± 1.5^{b}	33.4 ± 2.2^{C}	-0.2 ± 1.9	-0.7 ± 5.8	ns
dP/dt_{ma}	2261 ± 126^{b}	4253 ± 344^{A}	-1992 ± 265	-87.5 ± 9.3	0.000	1509 ± 86^{a}	2653 ± 153^{B}	-1144 ± 102	-76.8 ± 6.3	0.000
V_{max}	59.4 ± 3.1^{a}	87.1 ± 6.1^{A}	-20.5 ± 8.9	-36.7 ± 13.7	0.000	44.7 ± 2.4^{b}	62.3 ± 4.6^{B}	-17.6 ± 3.2	-39.2 ± 6.8	0.000
CO	1.87 ± 0.08^{ab}	1.84 ± 0.10^{AB}	0.04 ± 0.11	0.8 ± 5.8	ns	1.51 ± 0.08^{ac}	1.36 ± 0.10^{A}	0.15 ± 0.11	8.4 ± 6.8	ns
SV	16.8 ± 0.9^{a}	20.7 ± 1.5^{A}	-3.9 ± 1.1	-22.6 ± 7.2	0.004	14.6 ± 1.0^{ab}	16.7 ± 1.3^{AB}	-2.2 ± 0.7	-15.4 ± 4.5	0.004
AoPm	124 ± 4^{a}	144 ± 5^{A}	-19.7 ± 5.4	-16.9 ± 5.0	0.004	125 ± 6^{a}	149 ± 6^{A}	-23.7 ± 6.7	-21.1 ± 5.9	0.017
PaPm	18.0 ± 0.6^{a}	17.8 ± 0.7^{A}	0.2 ± 0.6	0.8 ± 3.4	ns	18.7 ± 1.1^{a}	18.2 ± 0.8^{A}	0.6 ± 0.6	2.0 ± 3.1	ns
SVR	65.3 ± 3.1^{a}	78.5 ± 5.0^{A}	-13.3 ± 5.0	-22.2 ± 7.9	0.022	83.4 ± 6.5^{a}	118 ± 15^{B}	-34.2 ± 12.1	-41.7 ± 13.2	0.017
ZaO	2.26 ± 0.08^{a}	2.85 ± 0.13^{A}	-0.59 ± 0.12	-27.1 ± 5.9	0.000	2.99 ± 0.29^{a}	4.02 ± 0.39^{A}	-1.03 ± 0.22	-38.0 ± 7.8	0.001
Zpa	0.82 ± 0.04^{a}	0.79 ± 0.05^{A}	0.03 ± 0.05	1.9 ± 6.4	ns	0.99 ± 0.08^{a}	0.96 ± 0.09^{A}	0.03 ± 0.06	2.0 ± 5.4	ns
PVR	5.2 ± 0.5^{a}	5.4 ± 0.6^{A}	-0.18 ± 0.51	-8.0 ± 9.0	ns	6.55 ± 0.90^{a}	5.31 ± 0.91^{A}	1.25 ± 0.61	23.2 ± 11.8	ns
RR	0.544 ± 0.026^{a}	0.724 ± 0.076^{AB}	-0.180 ± 0.068	-33.0 ± 11.7	0.023	0.577 ± 0.022^{a}	0.825 ± 0.125^{A}	-0.249 ± 0.107	-38.5 ± 14.5	0.040
PQ	0.096 ± 0.003^{ab}	0.108 ± 0.005^{AB}	-0.012 ± 0.005	-12.6 ± 5.0	0.028	0.111 ± 0.003^{a}	0.120 ± 0.003^{A}	-0.009 ± 0.003	-8.9 ± 2.5	0.004
QRS	0.043 ± 0.001^{a}	0.043 ± 0.002^{A}	-0.0004 ± 0.001	-1.5 ± 4.0	ns	0.047 ± 0.003^{a}	0.046 ± 0.003^{A}	0.001 ± 0.003	-0.7 ± 7.1	ns
QT	0.253 ± 0.006^{a}	0.254 ± 0.009^{A}	-0.002 ± 0.007	-0.6 ± 2.8	ns	0.258 ± 0.004^{a}	0.250 ± 0.007^{A}	0.008 ± 0.005	3.1 ± 1.8	ns
QTc	0.310 ± 0.004^{ab}	0.287 ± 0.007^{A}	0.023 ± 0.007	7.3 ± 2.3	0.009	0.310 ± 0.005^{ab}	0.274 ± 0.007^{A}	0.036 ± 0.005	11.7 ± 1.7	0.000
RP	154 ± 3^{a}	146 ± 4^{A}	7.7 ± 2.6	4.9 ± 1.6	0.013	159 ± 2^{a}	156 ± 3^{A}	2.7 ± 3.6	1.6 ± 2.3	ns

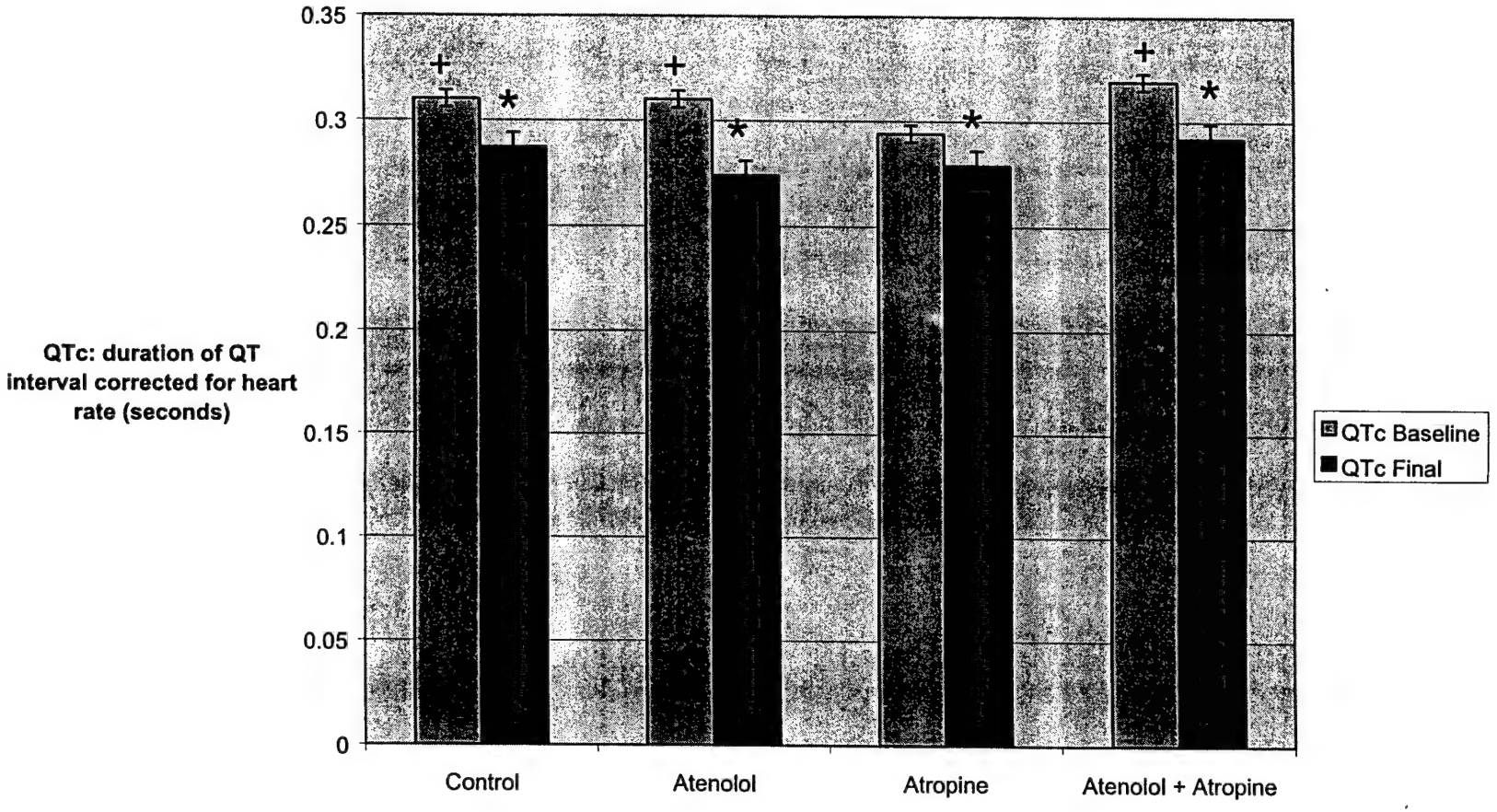
	atropine					atenolol+atropine				
	baseline	final	delta	delta%	P value	baseline	final	delta	delta%	P value
dose		52 ± 2^{A}					47 ± 2^{A}			
HR	174 ± 5^{b}	183 ± 7^{B}	-9.3 ± 9.0	-6.4 ± 5.3	ns	115 ± 3^{a}	129 ± 8^{C}	-13.4 ± 8.2	-12.3 ± 7.1	ns
LVEDP	3.9 ± 0.8^{b}	3.1 ± 0.7^{C}	0.8 ± 0.6	53.4 ± 25.6	ns	6.6 ± 0.7^{ab}	6.2 ± 0.8^{BC}	0.4 ± 0.9	-0.02 ± 13.0	ns
Ram	3.2 ± 0.8^{a}	2.9 ± 0.6^{A}	0.3 ± 0.4	-10.9 ± 37.3	ns	3.1 ± 0.3^{a}	2.9 ± 0.4^{A}	0.2 ± 0.2	9.5 ± 11.4	ns
tau	22.3 ± 0.9^{c}	19.2 ± 1.0^{A}	3.2 ± 0.7	14.3 ± 2.8	0.001	30.5 ± 1.2^{ab}	27.1 ± 1.2^{B}	3.4 ± 1.9	9.4 ± 5.9	ns
dP/dt_{ma}	2855 ± 161^{c}	5238 ± 456^{A}	-2383 ± 373	-84.0 ± 12.0	0.000	1472 ± 38^{a}	2871 ± 111^{B}	-1399 ± 92	-95.0 ± 6.0	0.000
V_{max}	71.9 ± 2.2^{c}	96.1 ± 8.4^{A}	-24.2 ± 8.0	-33.6 ± 9.9	0.011	48.9 ± 3.1^{ab}	69.0 ± 3.1^{B}	-20.1 ± 4.4	-46.0 ± 10.4	0.001
CO	1.97 ± 0.15^{b}	1.88 ± 0.15^{B}	0.09 ± 0.13	1.3 ± 8.1	ns	1.4 ± 0.1^{c}	1.6 ± 0.1^{AB}	-0.17 ± 0.04	-12.2 ± 3.1	0.003
SV	11.5 ± 0.9^{b}	10.5 ± 1.0^{C}	0.9 ± 1.1	2.4 ± 11.8	ns	12.2 ± 0.8^{b}	12.7 ± 1.2^{BC}	-0.5 ± 0.7	-3.8 ± 6.6	ns
AoPm	131 ± 4^{a}	149 ± 6^{A}	-18.7 ± 6.7	-15.2 ± 5.5	0.017	115 ± 5^{a}	150 ± 5^{A}	-35.6 ± 7.9	-34.7 ± 8.6	0.004
PaPm	17.0 ± 0.7^{a}	18.4 ± 0.8^{A}	-1.4 ± 0.8	-9.3 ± 4.7	ns	16.4 ± 0.5^{a}	18.2 ± 0.7^{A}	-1.8 ± 0.7	-11.7 ± 4.7	0.028
SVR	69.4 ± 6.4^{a}	84.0 ± 7.2^{AB}	-14.6 ± 6.4	-24.1 ± 8.7	0.045	83.6 ± 6.7^{a}	99.0 ± 7.4^{AB}	-15.4 ± 7.1	-23.0 ± 9.9	0.054
ZaO	2.72 ± 0.28^{a}	3.67 ± 0.35^{A}	-0.94 ± 0.34	-39.5 ± 11.3	0.017	2.70 ± 0.25^{a}	4.05 ± 0.38^{A}	-1.35 ± 0.39	-60.1 ± 18.5	0.005
Zpa	0.94 ± 0.10^{a}	1.18 ± 0.19^{A}	-0.24 ± 0.17	-26.5 ± 15.1	ns	0.86 ± 0.11^{a}	1.02 ± 0.15^{A}	-0.16 ± 0.11	-22.1 ± 14.2	ns
PVR	7.06 ± 0.83^{a}	9.01 ± 1.15^{A}	-1.95 ± 1.25	-34.9 ± 15.9	ns	7.51 ± 0.87^{a}	8.39 ± 1.10^{A}	-0.88 ± 0.79	-17.3 ± 14.3	ns
RR	0.349 ± 0.010^{b}	0.334 ± 0.013^{C}	0.015 ± 0.016	3.5 ± 4.7	ns	0.524 ± 0.012^{a}	0.481 ± 0.023^{BC}	0.043 ± 0.025	7.6 ± 4.9	ns
PQ	0.085 ± 0.004^{b}	0.094 ± 0.005^{B}	-0.009 ± 0.004	-11.6 ± 4.6	ns	0.103 ± 0.004^{a}	0.106 ± 0.004^{AB}	-0.003 ± 0.002	-2.9 ± 2.1	ns
QRS	0.041 ± 0.001^{a}	0.043 ± 0.002^{A}	-0.002 ± 0.002	-4.3 ± 4.1	ns	0.046 ± 0.003^{a}	0.042 ± 0.001^{A}	0.004 ± 0.003	5.1 ± 4.1	ns
QT	0.208 ± 0.007^{b}	0.193 ± 0.005^{B}	0.014 ± 0.007	6.0 ± 3.5	0.058	0.257 ± 0.003^{a}	0.228 ± 0.005^{A}	0.029 ± 0.005	11.3 ± 2.1	0.000
QTc	0.294 ± 0.008^{b}	0.279 ± 0.006^{A}	0.015 ± 0.007	4.7 ± 2.5	0.050	0.319 ± 0.003^{a}	0.292 ± 0.005^{A}	0.028 ± 0.003	8.7 ± 1.0	0.000
RP	128 ± 3^{b}	125 ± 2^{B}	2.9 ± 3.2	1.7 ± 2.2	ns	155 ± 3^{a}	148 ± 2^{A}	7.3 ± 1.7	4.6 ± 1.1	0.002
									values=mean ± SE	

Delta refers to the absolute difference between baseline (prexposure to ouabain) and pre-ectopy (the value just before ventricular ectopy developed). Delta % refers to the percent change between baseline and pre-ectopy. Values for baseline followed by the small case letter does not differ for baseline among groups. Values for pre-ectopy followed by the same capital case letter does not differ for pre-ectopy among groups. P values for differences between baseline

and pre-ectopy are shown in the right hand columns for each group. The dose of ouabain required to produce ventricular ectopy for each group is shown.

HR: heart rate; LVEDP: left ventricular end-diastolic pressure; PRA: mean right atrial pressure; dP/dtmax: maximal rate of increase in left ventricular pressure; CO: cardiac output; SV: stroke volume; PAO: mean aortic pressure; PPA: mean pulmonary pressure; SVR: systemic vascular resistance; ZAO: estimate of systemic vascular impedance; ZPA: estimate of pulmonary vascular impedance; PVR: pulmonary vascular resistance; RR: duration of R-R interval; PQ: duration of PQ interval; QRS: duration of QRS complex; QT: duration of QT interval; QTc: duration of QT interval corrected for heart rate; RP: ventricular refractory period

Figure 1: QTc recorded during baseline and preceding ventricular ectopy



* Significant difference from baseline to final $p \leq 0.05$
+ Significant difference from atropine baseline $p \leq 0.05$

(1) dP/dtmax is the maximal rate of decrease in intraventricular pressure occurring during isovolumetric contraction. This value increases with increases in either myocardial contractility or preload, but is used often as an index of contractility.

(2) Tau is defined as the time required for intraventricular pressure to decrease 63% of the value from the maximal rate of decrease to a level 10 mmHg above EDP. Tau is thought to reflect the time required for resequestration of Ca++ from troponin-C to the sarcoplasmic reticulum.

(3) A plot of dP/dt/kP on the ordinate versus intraventricular pressure on the abscissa normally results in a straight line during the period of isovolumetric contraction. The extrapolation of the straight line to the X-axis intercept, Po, intercepts the Y-axis at a point representing the maximal velocity of fiber shortening achieved at 0 load-a load-independent index of contractility. The _ stiffness constant, k, is neglected, therefore the units on the abscissa-which should be muscle lengths/second, are relative.

(4) Impedance refers to the interference to the flow of blood from the LV to the Ao, and is caused, principally, by the stiffness of the aorta. Correct units of impedance require complex mathematical assumptions and calculations, but the ratio of pulsatile pressure to pulsatile flow (SV) is a reasonable approximation.

(5) Normally when pacing of the ventricle stops, there is no continuation of ectopic depolarizations, termed repetitive ventricular responses (RVR). When RVR's occur, there is indication of increased ventricular irritability.

(6) Each stimulus was of 2 ms duration and of a magnitude twice the mid-diastolic threshold.

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A LOGISTIC REGRESSION MODEL TO PREDICT ONSET OF CARDIAC ARRHYTHMIA

ABSTRACT

The purpose of this investigation was to determine if a mathematical model could be constructed to predict the onset of cardiac arrhythmia. The dog and pig were used as surrogates for the human. The dog was measured both awake and under anesthesia while the pig was only measured under anesthesia.

Recordings were made during a control period and after exposure to repeated doses of ouabain, a digitalis glycoside known to provoke ventricular arrhythmias. The construction of the model was based on logistic regression because such a model would permit the user to predict the probability of developing an arrhythmia for a given value of the observed parameter.

Our investigation found arrhythmia was predictable in dogs, both awake and anesthetized. In both the dog and pig, the onset of arrhythmia was predicted by shortening of the QT interval, increase in systolic aortic pressure, increase in dP/dT_{max}, and prolongation of the PQ interval.

This study demonstrated a model can be constructed to predict the onset of arrhythmia induced by varying doses of ouabain, in both the awake and anesthetized dog and in the anesthetized pig. The model allows for the prediction of the probability of onset of arrhythmia for a given value of specific variables. It is proposed this same methodology could be used to develop a model for use in humans when exposed to various arrhythmia-producing compounds (e.g. halons).

Introduction

Cardiac sensitization is known to occur when an exogenous chemical causes hyper sensitization of the cardiac muscle; thereby, predisposing the heart to ventricular arrhythmia upon the release of epinephrine. Cardiac arrhythmia can occur in humans during stressful situations ranging from evacuating a burning building to battlefield conditions. Under these types of conditions epinephrine is released. Adrenaline triggers the ventricular arrhythmia, causing a heart attack. Stressful situations alone are not enough to produce an arrhythmia; an exogenous chemical must be present. A partial list of chemicals that may have the potential to produce arrhythmia include halogenated hydrocarbons (refrigerants and fire extinguishers), digitalis glycosides and cocaine.

Cardiac sensitization tests currently accepted by the federal regulators are conducted using dogs, which are exposed to the chemical of interest in combination with epinephrine challenge[1]. In such tests, the animals are exposed first to an epinephrine challenge, followed five minutes later

by an exposure via inhalation to a test chemical for five minutes. Then the animals are challenged with epinephrine and monitored while the exposure continues for an additional five minutes. During the exposure period the electrocardiogram is monitored for cardiac arrhythmia. When the dog model was developed, the development of an arrhythmia was the only defined and acceptable endpoint by which to measure cardiac sensitivity. However, scientific advances and the view of the general public have rendered the model rather extreme.

The overall aim of this research was to construct a mathematical model of the physiological threshold response(s) that drive(s) the heart toward arrhythmia. This response may be a mechanical, biochemical, electrophysiological or a combination of these processes. For this study ten mechanical/hemodynamic parameters were measured just prior to the development of an arrhythmia. As a result of these studies a set of parameters have been identified that predict the onset of ouabain-induced arrhythmia before life threatening electrophysiological and hemodynamic changes occur.

Materials and Methods

Experiment 1: Animals

Six male (10 kg) beagle dogs.

Physiological Parameters

A combination of hemodynamic and electrophysiological parameters was measured during a baseline state (control), after exposure to graded doses of ouabain and immediately prior to onset of arrhythmia. The parameters are as follows:

1) PQ interval, sec

2) QT interval, sec

3) QTc (QT corrected for HR), interval, sec (added in experiment 2)

4) dP/dT_{max}, mm or mmHg/s

5) AoPs (systolic aortic pressure), mmHg

6) AoPd (diastolic aortic pressure), mmHg

7) AoPm (mean aortic pressure), mmHg

8) LVED (left ventricular end-diastolic pressure)

9) CO (Cardiac Output)

10) SVR (AoPm/CO)

Pulsatile aortic (AP) and left ventricular pressures (LVP), dP/dT, and pulsatile aortic flow, were recorded on a direct-writing photographic oscillograph and FM tape. Systemic vascular resistance was estimated by dividing mean systemic arterial pressure (presuming right atrial pressure was constant) by cardiac output. To estimate contractility, dP/dT_{max} was used, realizing that this method is load dependent. ECGs were analyzed for heart rate, and PQ, and QT durations.

Chemical/Drug

All animals were dosed intravenously, with ouabain – a rapid-acting digitalis glycoside. This compound is known to produce cardiac arrhythmia; hence it was a logical chemical to use to construct a mathematical model.

Instrumentation

The experimental design was adopted from Robitaille, *et al.*[2], 1993; Rath, *et al.*[3], 1995; and Josephson[4], 1992. Prior to the start of the study, each dog was anesthetized with thiopental-halothane. Catheter-introducers were placed in a jugular vein and a carotid artery. Animals were allowed to recover after the surgery. Prior to exposure to ouabain, a pacing catheter was introduced through the jugular vein with the bipolar leads being positioned in the right ventricle. A Millar catheter containing two solid-state pressure transducers and a flow meter was introduced through the carotid artery. One of the pressure transducers was positioned in the left ventricle and the other in the ascending aorta with the flow meter. Electrodes forming lead II ECG were placed on the limbs of the animals. During the first phase of the dog study, the parameters were measured in conscious dogs. Each animal was dosed, intravenously, with 40 μg/kg ouabain (priming dose). Additional doses of 5 μg/kg ouabain were given every 15 minutes (graded doses) until ventricular arrhythmia was produced. All parameters were measured

during the baseline period (control), after infusion of the priming dose and after each graded dose. Before each ouabain infusion, the right ventricle was paced ten times (eight conditioning stimuli at 300 ms intervals, the 9^{th} stimulus at 150 ms interval, and the 10^{th} stimulus at 130 ms interval) to determine if non-paced ventricular ectopic activity (termed RVR for repetitive ventricular responses) occurred. The end-point of each experiment was taken when the dog developed the first ventricular premature depolarization, either spontaneously or after cessation of pacing. For the second phase of the dog study, the same animals were anesthetized 24-hours later. The dogs were anesthetized intravenously with thiopental sodium (15 mg/kg) and alpha chloralose (100 mg/kg), and measurements were made as in the first phase. Upon completion, the dogs were euthanized before awakening.

Statistical Methods

These data were evaluated using logistic regression since the outcome variable is binary[5]. The outcome variable was defined as baseline or prior to arrhythmia. The analysis was performed using the logistic

procedure in SAS Version 7.0. The independent variables in the model for evaluating the effect of anesthesia on dogs were the parameters measured and 'Group' i.e. conscious or anesthetized. The dependent variable was assigned a value of 'zero' for control or a value of 'one' for the onset of arrhythmia. A significance level of 0.10 was chosen as an inclusion criterion, since this study was to investigate the feasibility of building a predictive model. Logistic regression is given by equation 1 with p being the probability of arrhythmia, X_1 the measurement of the corresponding cardiac parameter, and β_0, β_1 and β_2 are the parameters of the logistic function to be estimated. Group, X_2, is given a value of 'one' for conscious or 'two' for anesthetized.

The output from SAS includes the β_0, β_1, and β_2 estimates, p-value for these estimates, and a p-value for assessing goodness-of-fit for the model using -2 Log Likelihood as the criteria, which has a chi-square distribution under the null hypothesis.

Experiment 2: Animals

Twelve male (10kg) beagle dogs and twelve male (25kg) Yorkshire swine were used.

Physiological Parameters

The parameters were measured were the same as in the experiment 1.

Chemical/Drug

The dogs and pigs were treated as the anesthetized dogs in the first experiment. In this study the logistic regression equation is simplified from that of Experiment 1 and is given by equation 2,

where p is the probability of arrhythmia, X_1 the corresponding cardiac parameter measurement, and β_0 and β_1 are the parameters of the logistic function to be estimated.

Results

Experiment 1

The first step in evaluating the model was to examine the p-value for the goodness-of-fit. Those parameters which exhibited a significant goodness-of-fit included PQ, QT, AoPs, and $dP/dT_{max.}$ Table 1 contains these results. Further examination showed that the models for PQ, QT, AoPs and dP/dT_{max} were significant with respect to the coefficient of these parameters. These same models were not significant with respect to the "Group" factor, indicating the responses in conscious and anesthetized dogs were similar. Twenty-two observations were made during this study; these included two readings (baseline and prior to arrhythmia) for five conscious animals and two readings (baseline and prior to arrhythmia) for six anesthetized animals. Due to technical difficulties, the values for one animal during the conscious phase of the study are missing.

Experiment 2

Table 2 contains the results of the second experiment. The entries in this table are the p-values of the coefficient of the respective parameters, with $p<0.1$ being considered significant. Figures 1-3 illustrate the logistic regression curves for each of these significant parameters for both dog and swine.

To illustrate the predictive properties of the model, Figures 4-11 show all data points for each subject from baseline to the onset of arrhythmia along with lines indicating the 0.50 and/or 0.80 probability levels for the onset of arrhythmia. Time was normalized to a 0 to 1 scale with 0 being baseline and 1 being just prior to the onset of arrhythmia. It was necessary to normalize time because some subjects developed arrhythmias from the ouabain exposure more quickly than others. Figures 4,5,8 and 9 show the similarity of the response of dP/dT_{max} to the insult of ouabain in the dog and pig. Figures 6 and 7 show the response of QTc in the pig. Figures 10 and 11 show the response of AoPs in the dog.

Discussion

The objective of this investigation was to construct a mathematical model which can predict the onset of cardiac arrhythmia due to exposure to ouabain. The model is based upon physiological measurements at baseline and prior to the onset of cardiac arrhythmia. In constructing the model, the focus was to develop a bridge between the current dog model and a mathematical model. The current model uses conscious dogs, but the method that is used to acquire the physiological data would be more humane if the dogs were anesthetized, thus is was necessary to determine if anesthesia exerted a statistically significant effect on the measured parameters. Comparing data from conscious dogs to anesthetized dogs indicates that anesthesia has no significant effect on the physiological parameters of concern, as seen by the lack of significance for the factor 'Group' in Table 1.

Logistic regression was used to develop these models because of the binary nature of the outcome (baseline/onset of arrhythmia). It provides an estimate of the probability of a dog or pig having a value of a

parameter which occurs just prior to the onset of cardiac arrhythmia, whereas conventional inferential statistics would only determine if control values for each parameter are significantly different from their corresponding values at the onset of arrhythmia. The only limitation of logistic regression is the event of all control values being contained in an interval such that there is no overlap with the interval containing the impending onset values. In this situation, there is no single logistic function, which will describe the data; instead there are an 'infinite' number of functions which could describe the data.

In the dog for Experiment 2, several parameters were found to be significant predictors of onset of cardiac arrhythmia. Three of these parameters were AoPs, QTc, and dP/dT_{max} and each has a unique function that can be considered as an independent model for predicting the onset of cardiac arrhythmia. Combinations of these parameters did not yield a model with better predictive power than the single variable models.

In the pig, Experiment 2 found three of the same parameters as in the dog were significant predictors of onset of cardiac arrhythmia. The parameter dP/dT_{max} did not have a unique model because of complete separation of the baseline and onset values, see Table 2 and Figure 1. Pigs were also used as an animal model to confirm the findings in the dog as well as being a transition model for humans, see Figures 1-3.

We have shown that a logistic regression model to predict the onset of arrhythmia can be constructed for either the dog or pig. Several parameters, AoPs, QTc, and dP/dT_{max} are significant predictors of ouabain-induced cardiac arrhythmia in both pigs and dogs, see Figures 1-3 and Table 2.

This logistic regression model used baseline data and the data immediately prior to the onset of arrhythmia. The temporal nature of the change in parameters from baseline to onset of arrhythmia was investigated. Figures 4 - 11 show that each predictor parameter changes in essentially a monotonic fashion from baseline to the onset of arrhythmia. Thus after any statistically significant parameter reaches a

critical value, the dog or pig is at increased risk for the onset of a cardiac arrhythmia. A critical value for a given parameter is determined by the investigator as the value of the parameter at which there is concern for the safety of the subject. For example, in Figure 8, a value of dP/dT_{max} above 3200 has probability of 0.8 of being in the range of values which occur just prior to the onset of arrhythmia. Continuous monitoring would provide warnings that the value of a given parameter was approaching the value associated with the onset of arrhythmia.

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Acknowledgements

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The Experiments reported here were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, DHHS, Publication NO (NIH) 86-23 (1996).

Table 1. Results of Multiple Logistic Regression: p-values for the Coefficients of the Variables Predicting Cardiac Arrhythmia in Conscious and Anesthetized Dogs.

Variable	Intercept (β_0) p-value	Parameter (β_1) p-value	Group (β_2) p-value	GOF* p-value
PQ	**0.05**	**0.04**	0.21	**<0.01**
QT	**0.04**	**0.02**	0.20	**<0.01**
dP/dTmax	**0.03**	**0.02**	0.07	**<0.01**
AoPs	**0.06**	**0.05**	0.41	**0.01**
AoPd	0.45	0.34	0.69	0.62
AoPm	0.16	0.10	0.50	0.18
LVED	0.43	0.15	0.71	0.29
CO	0.23	0.08	0.79	0.17
SVR	0.54	0.06	0.56	0.09

* = Goodness-of-fit (GOF) for the model using -2 Log Likelihood as the criteria which has a chi-square distribution under the null hypothesis.

Table 2. Results of Logistic Regression. Values given are p-values for the test of significance for the coefficient of the independent variable predicting onset of arrhythmia in dogs and swine.

Variable	Pig (N=12)	Dog (N=12)
AoPs	**0.0332**	**0.0111**
QTc	**0.0332**	**0.0290**
QT	**0.0114**	0.8997
PQ	**0.0532**	0.0693
dP/dT_{max}	*******	**0.0545**
AoPm	0.1455	**0.0180**
SVR	0.4006	**0.0543**
AoPd	0.5027	**0.0419**
LVED	0.755	0.7866
CO	0.7763	0.7480

**** indicates baseline and onset values are non-overlapping sets and hence no unique function applies.

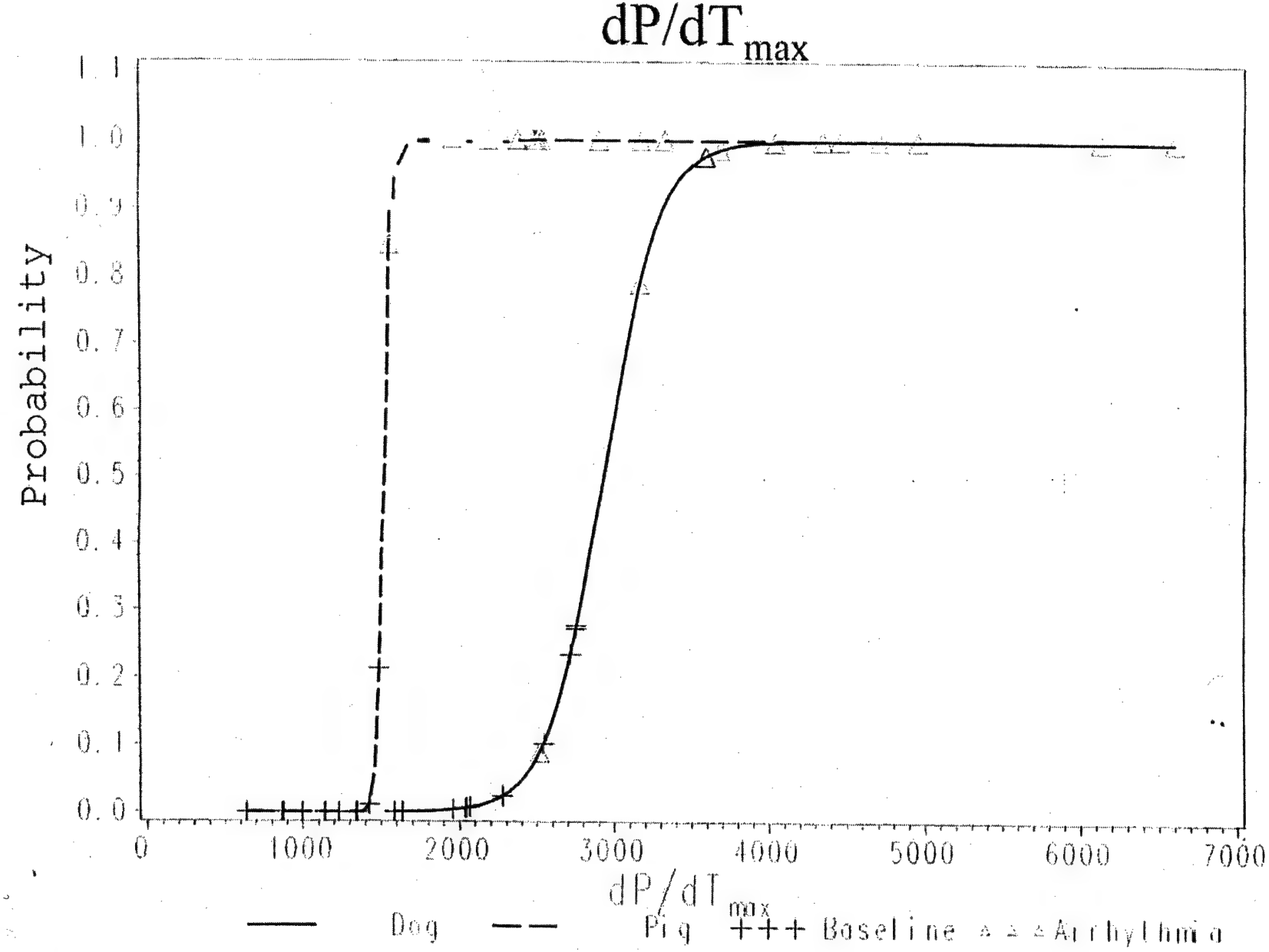
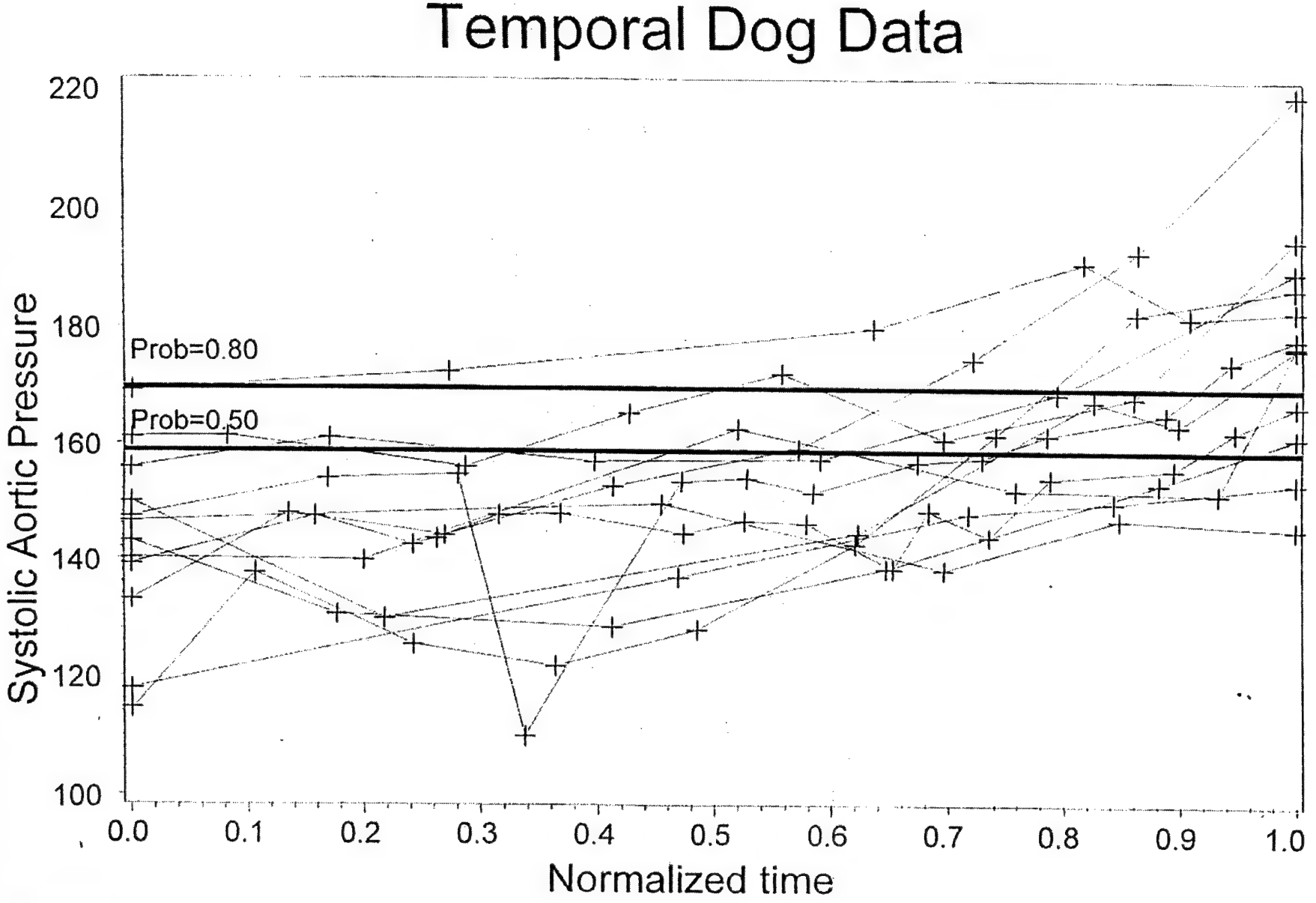


Figure 1
E. Herderick

$$p = \frac{e^{\beta_0 + \beta_1 X_1}}{1 + e^{\beta_0 + \beta_1 X_1}} \qquad \text{Equation 2}$$



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E. Herderick

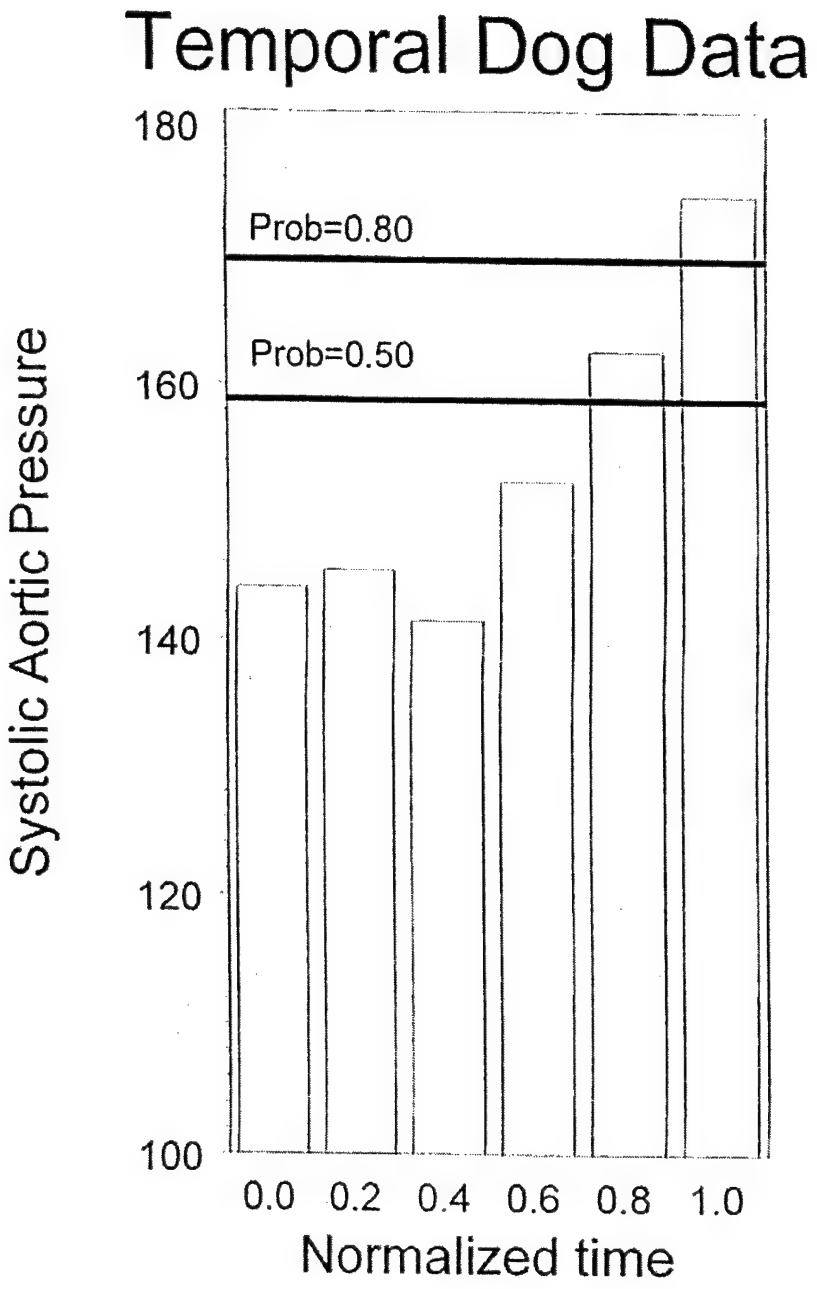
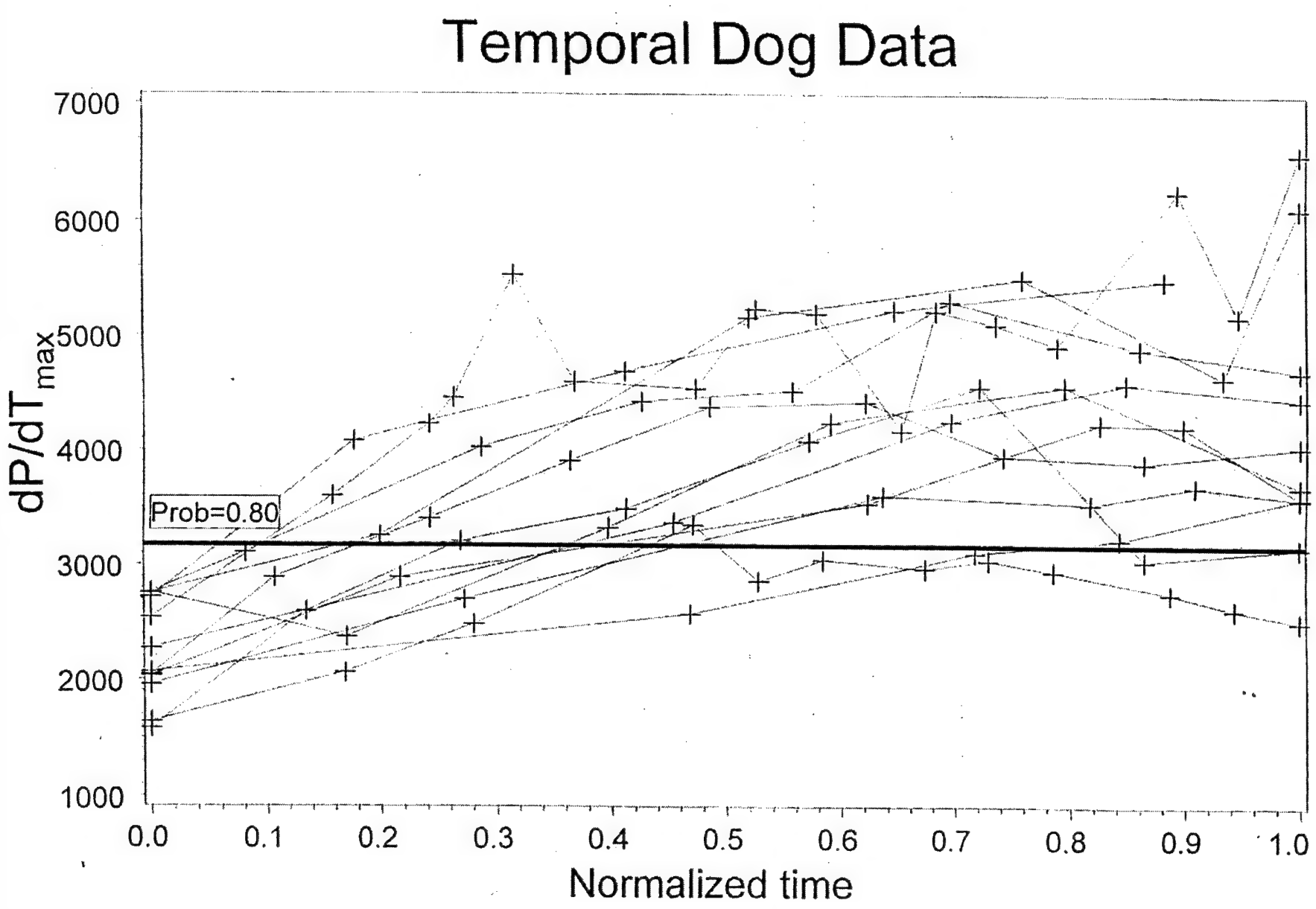


Figure 10
E. Herderick



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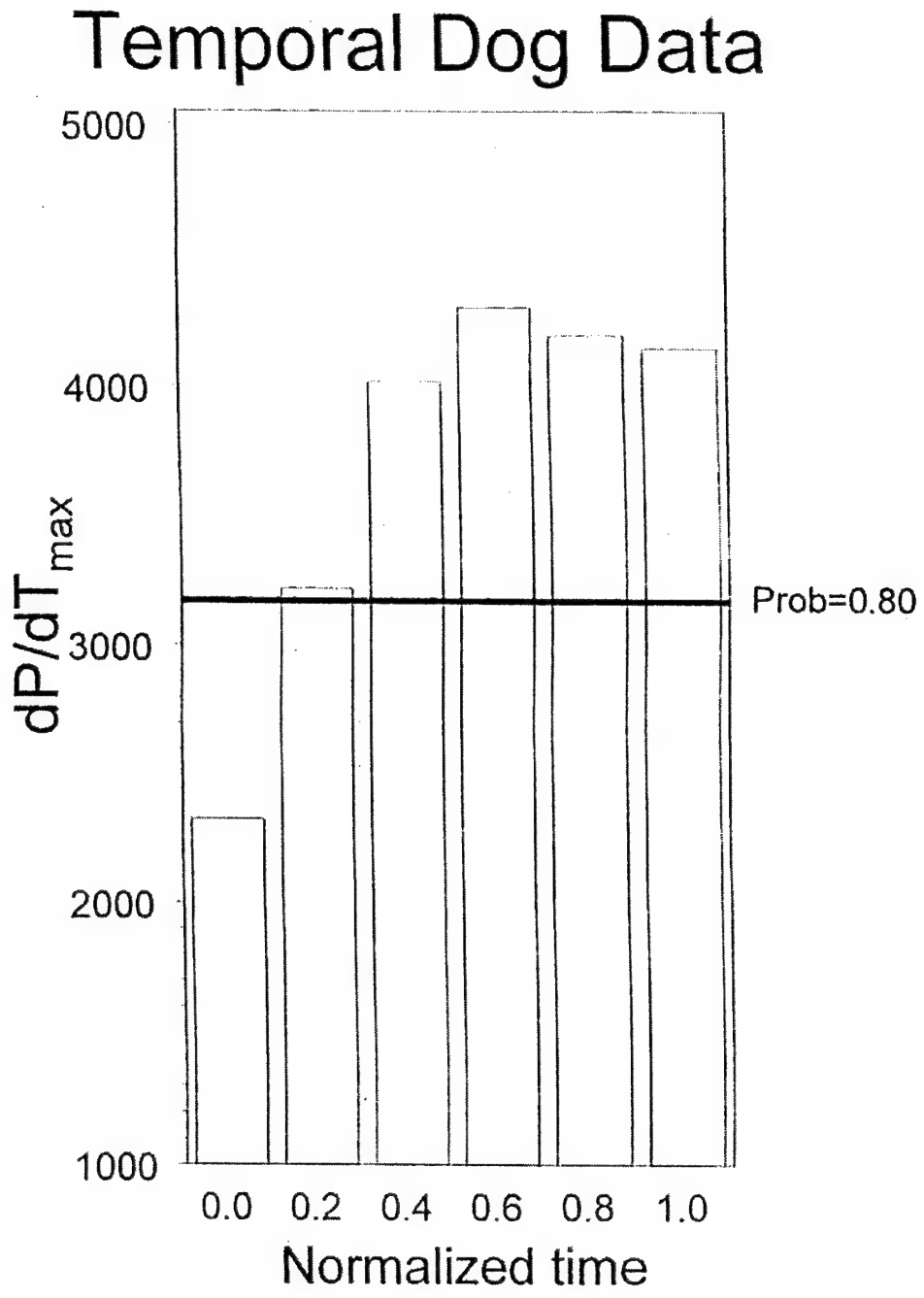
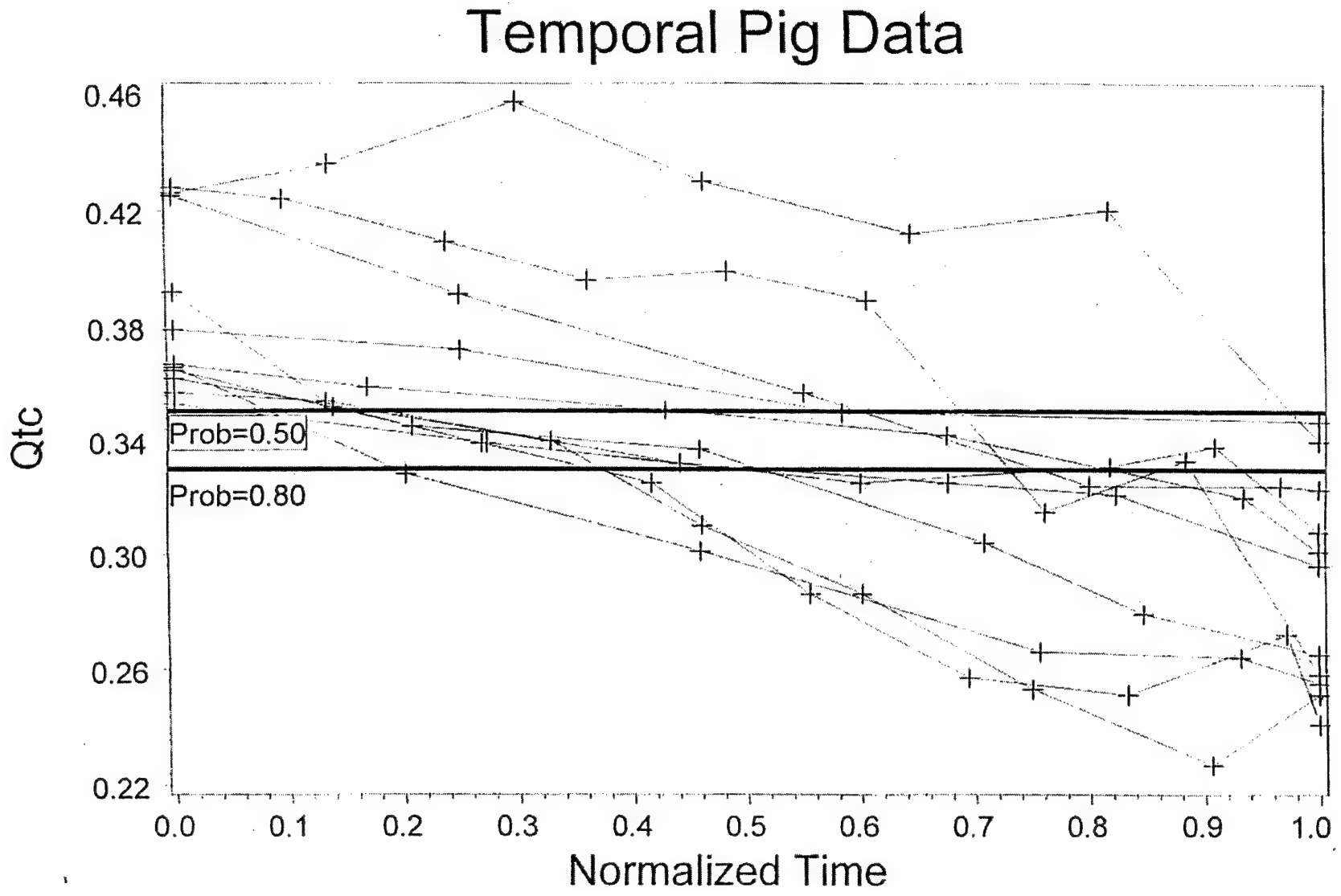


Figure 8
E. Herderick



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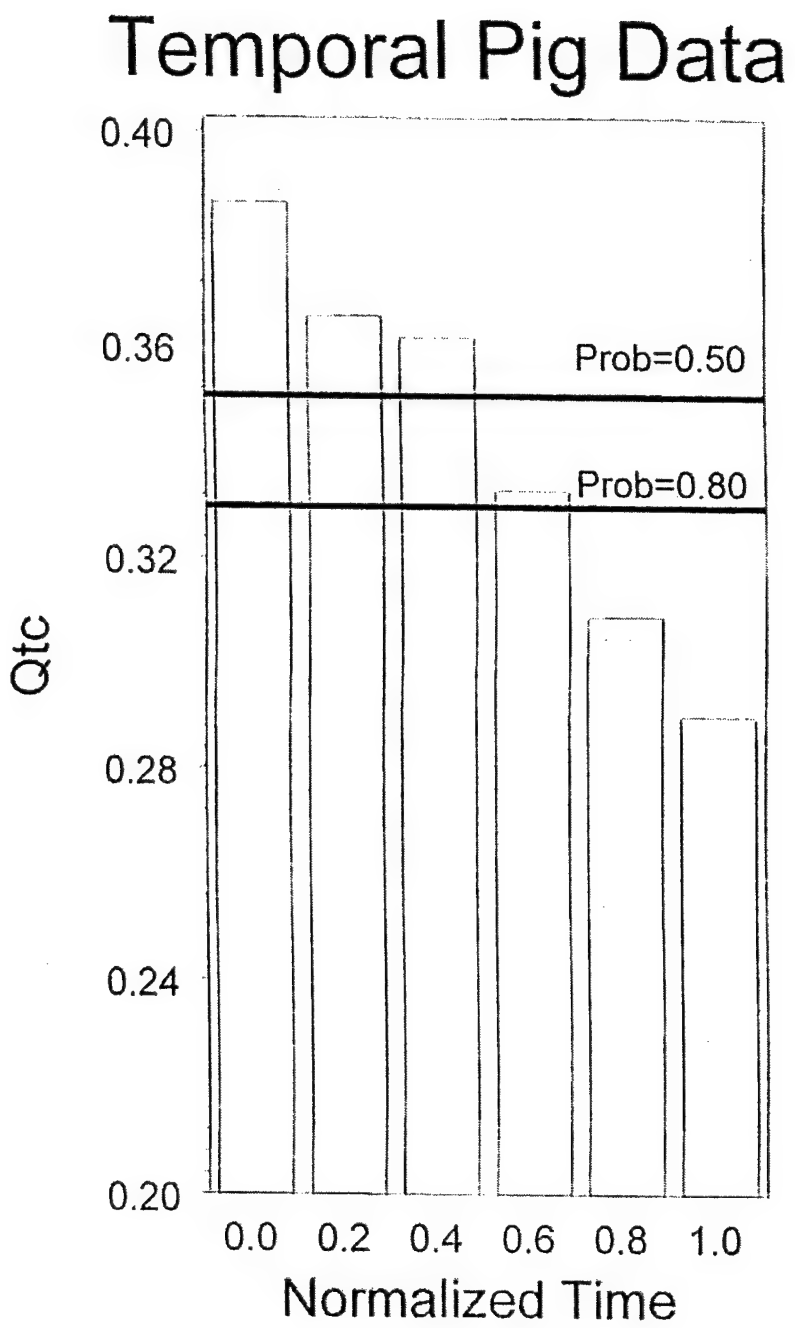
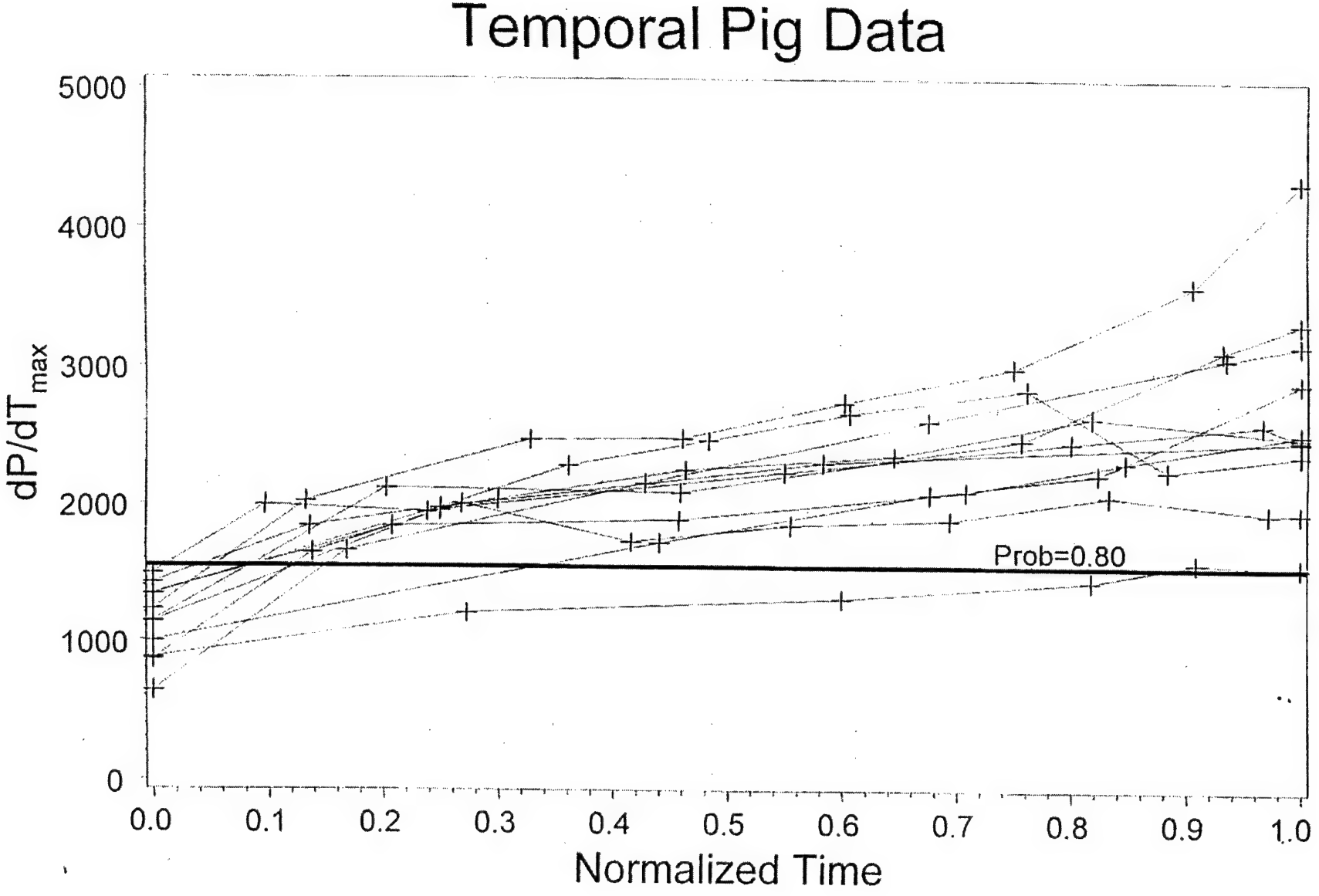


Figure 6
E. Herderick



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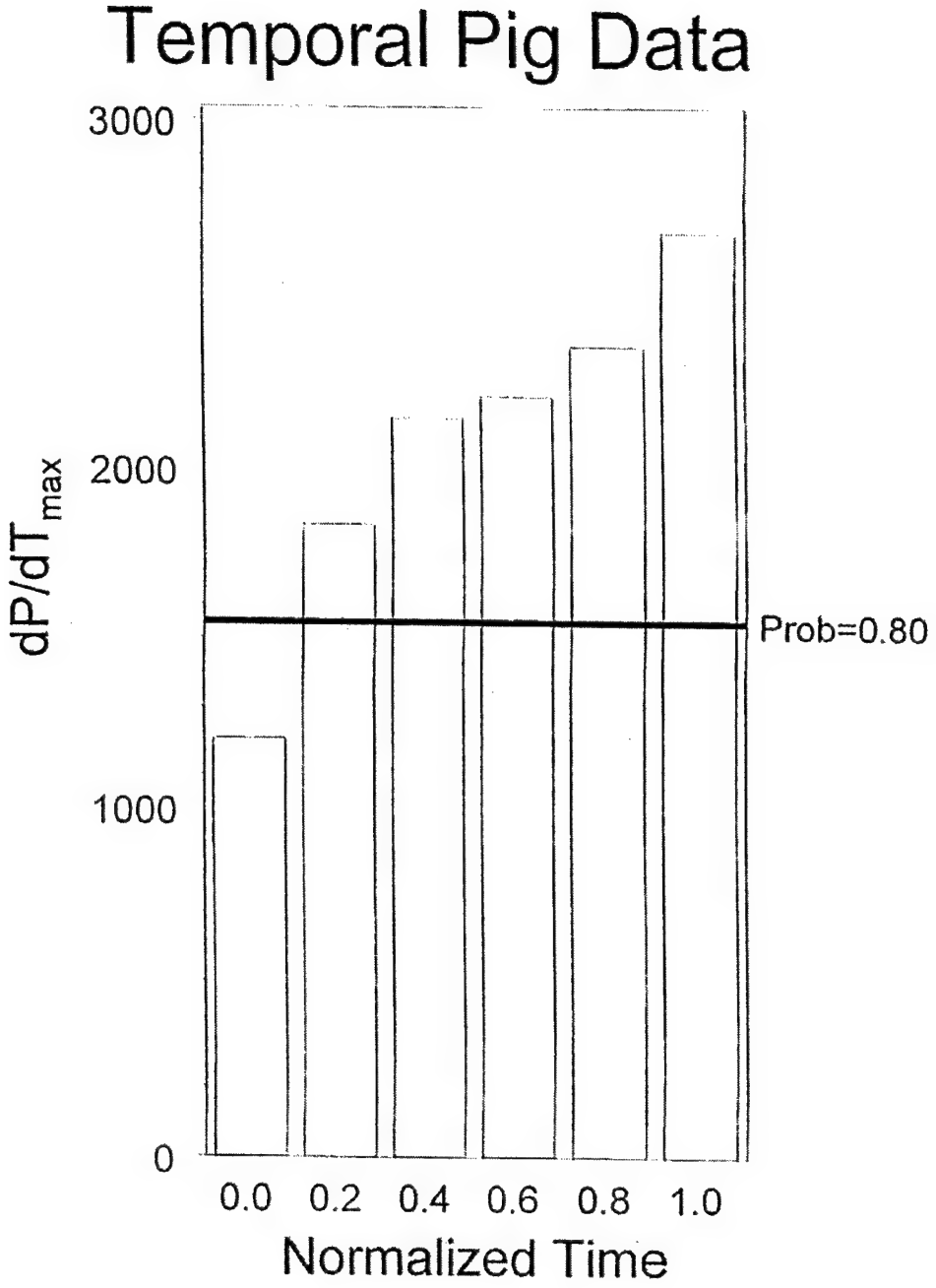

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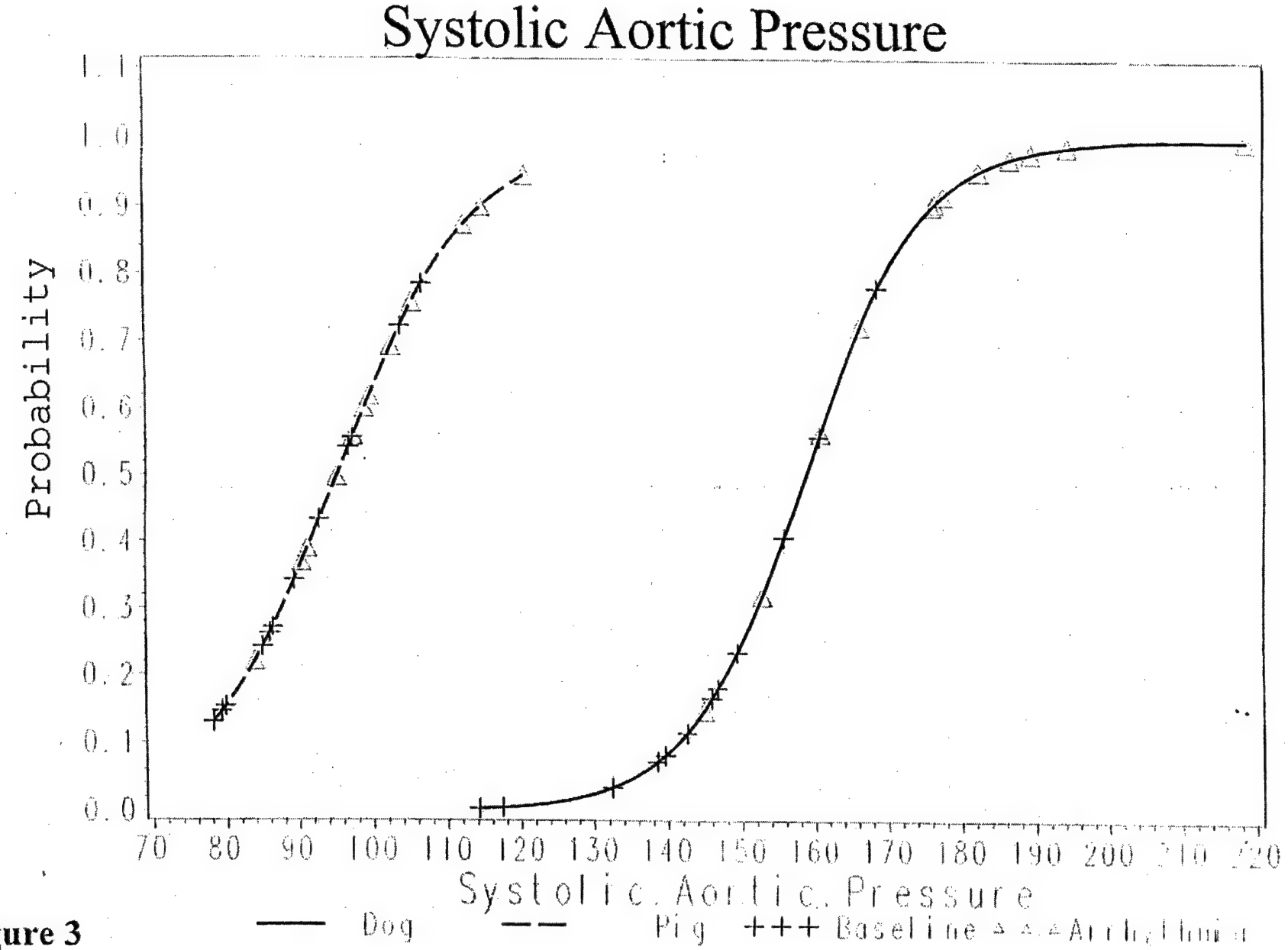

Figure 3
E. Herderick

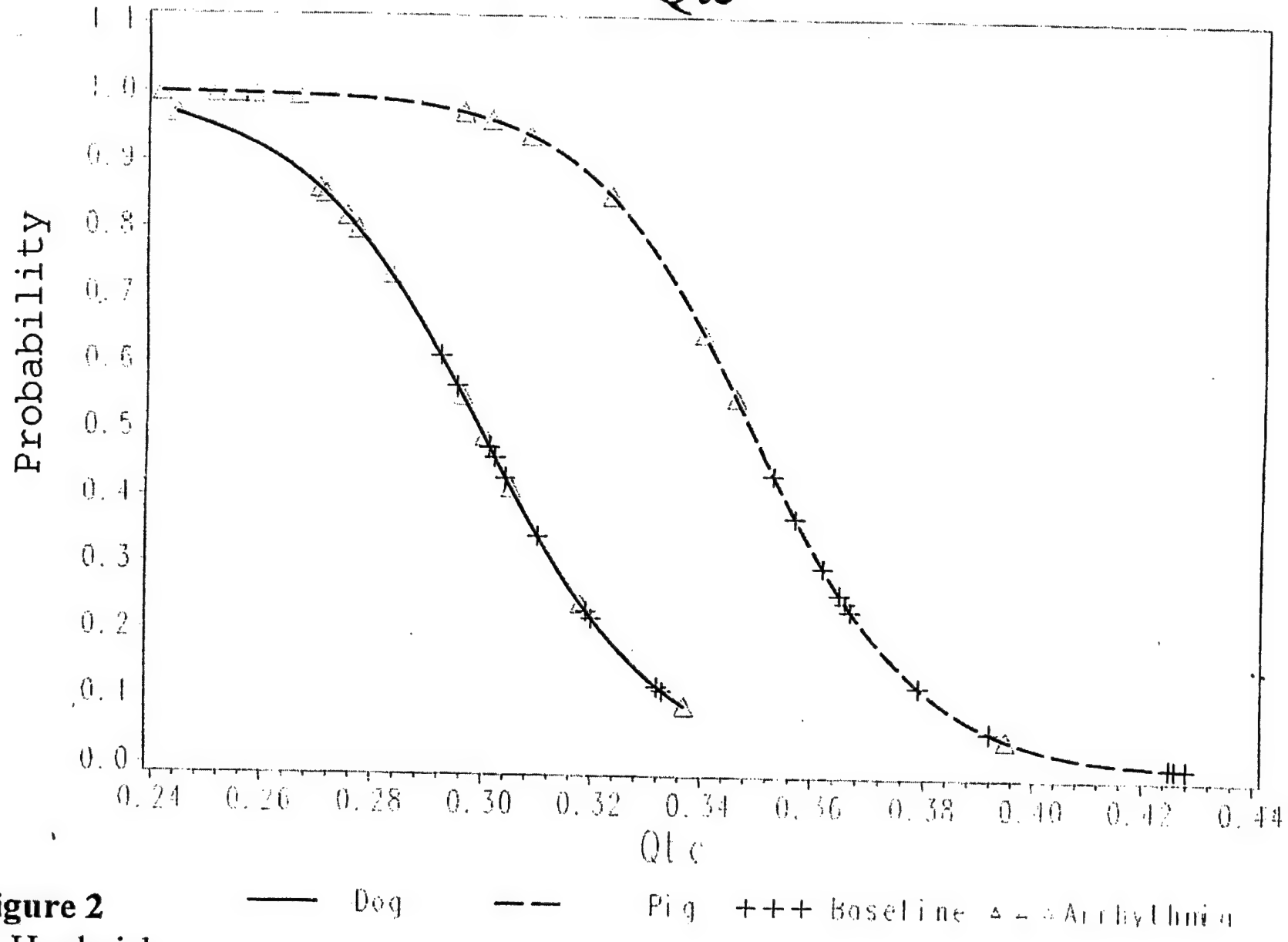

Figure 2
E. Herderick

$$p = \frac{e^{\beta_0 + \beta_1 X_1 + \beta_2 X_2}}{1 + e^{\beta_0 + \beta_1 X_1 + \beta_2 X_2}} \qquad \text{Equation 1}$$

A New Perspective for Identifying Potential Cardiac Sensitizers.

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A New Perspective for Identifying Potential Cardiac Sensitizers. Smith, E., Nakayama, T., Herderig, E., Powers, J., Briggs, G., Still, K., and Hamlin, R. (1999) *Toxicol. Sci* ( )

Abstract

The purpose of this investigation is to determine if a mathematical model can be constructed for predicting the onset of cardiac sensitization. Both the dog (awake and anesthesia) and the pig (anesthesia) were used as surrogates for the human. Physiologic and electrocardiographic measurements were taken during the control period and after each exposure to graded doses of oubain, a digitalis glycoside known to provoke ventricular arrhythmia. The construction of the model was based on logistic regression because by the nature of the procedure such a model would permit the observer to predict the probability of developing an arrhythmia for a given value of the observed parameter. Arrhythmia was predictable in dogs, both awake and anesthetized, by a reduction of heart rate, prolongation of the PQ interval, lengthening of the QT duration, increased arterial pressure and increased maximal rate of pressure development by the left ventricle dP/dtmax. In pigs, the onset of arrhythmia was predicted by shortening of the QT interval, prolongation of the PQ interval and by increased in dP/dtmax. This study demonstrated a model could be constructed to predict the onset of arrhythmia induced by varying doses of ouabain, in both the awake and anesthetized dog and in the anesthetized pig. The model enables the user to predict the probability of arrhythmia with a given change in the specific variables. The anticipated result of this methodology is the fast and reliable creation of a mathematical model for predicting potential cardiac sensitizer with out the use of animal research.

Key Words: Cardiac Sensitization, Ventricular Arrhythmia, Dogs, Swine, Ouabain,

Introduction

Cardiac sensitization is a condition that does not lend itself well to definition. In its simplest terms it occurs when an exogenous chemical causes hypersensitization of the cardiac muscle: thereby, predisposing the heart to ventricular arrhythmia upon the release of epinephrine. Cardiac sensitization occurs during stressful situations, where an individual is undergoing a "Fight or Flight" response. Stressful situations range from evacuating a burning building to battlefield conditions. During the "Fight or Flight" response, epinephrine is released. Adrenaline triggers the ventricular arrhythmia, causing a heart attack. Stressful situations alone are not enough to produce a cardiac sensitization; an exogenous chemical must be present. Chemicals that have a cardiac sensitizing potential include halogenated hydrocarbons (refrigerants and fire extinguishers), digitalis glycosides and cocaine.

Cardiac sensitization tests currently accepted by the federal regulators are conducted using dogs, which are exposed to the chemical of interest in combination with epinephrine challenge (U.S. EPA 1994). In such tests, the animals are exposed first to an epinephrine challenge, followed 5 minutes later by an exposure via inhalation to a test chemical for 5 minutes. Then the animals are challenged with epinephrine and monitored while the exposure continues for an additional 5 minutes. During the exposure period the cardiac electrical activity in each animal is monitored for cardiac arrhythmia. When the dog model was developed, the observation of arrhythmia was the only known and acceptable endpoint (parameter) by which to measure cardiac sensitivity. However, scientific advances and the view of the general public have rendered the model rather extreme and the endpoint (arrhythmia) controversial.

Even though several *in vitro* models have been attempted, it is apparent that a better understanding of the mechanism that produces cardiac sensitization is still needed. The overall aim of this research is to isolate the physiological threshold response(s) that drive(s) the heart toward arrhythmia. This response may be a mechanical, biochemical, electrophysiological, or combination of these processes. For this study 15 mechanical or hemodynamic parameters have been evaluated prior to the development of arrhythmia. As a result of these studies a series of parameters have been identified that will accurately predict potential cardiac sensitizers (chemicals) before life threatening electrophysiological and hemodynamic changes occur.

Methods and Materials

Animals: Six, young-mature, healthy, male beagle dogs and six, healthy, 35 kg, male Yorkshire swine were used

Physiological Parameters: A combination of hemodynamic and electrophysiologic parameters were measured, during a control state and following graded concentrations of ouabain used to provoke ventricular arrhythmia. The parameters are as follows:

1) HR, beats/minute
2) PQ interval, sec
3) QT interval, sec
4) $dP/dt_{max.}$, mm or mmHg/s
5) $dP/dt_{min.}$, mm or mmHg/s
8) Paom (mean aortic pressure), mmHg
9) LVED (left ventricular end-diastolic pressure)
10) Peak (peak aortic blood flow), cm/sec
11) DT (duration time of ejection), sec
12) TVI (time velocity interval), cm

6) Paos (systolic aortic pressure), mmHg

7) Paod (diastolic aortic pressure), mmHg

13) CO, TVI*HR

14) Z, dPao/dF

15) SVR (Poam/CO)

Pulsatile aortic (AP) and left ventricular pressures (LVP), dLVP/dt, pulsatile aortic flow and its integral (SV) were recorded on a direct-writing photographic oscillograph and FM tape. Aortic impedance was estimated by dividing pulsatile aortic pressure by pulsatile flow (i.e. stroke volume), and systemic vascular resistance was estimated by dividing mean systemic arterial pressure (presuming right atrial pressure was constant) by cardiac output. dP/dt_{max} and dP/dt_{min} were used to estimate contractility and lusitropy, respectively. ECGs were analyzed for heart rate (chronotrope), and PQ (dromotrope) and QT (repolarization) duration.

Chemical/Drug: All animals were dosed, i.v., with ouabain, a rapid-acting digitalis glycoside and know cardiac sensitizer. Pigs were an anesthetized with thiopental and halothane; and dogs with thiopental-chloralose.

Experimental Design: The primary objective of this investigation was to determine if it is possible to construct a mathematical model to predict the onset of cardiac arrhythmia. A corollary to this objective is to determine if anesthesia in any way obfuscates the ability of physiological monitoring to predict the onset of arrhythmia.

Dog Study: The experimental design was adopted from Robitaille, *et. al.*, 1993; Rath, *et. al.*, 1995; and Josephson, 1992. Prior to the start of the study, each dog was anesthetized with thiopental and halothane, and catheter-introducers were placed in a jugular vein and a carotid

artery. Animals were allowed to recover after the surgery. Prior to exposure to the arrhythmogenic compound (ouabain), a pacing catheter was introduced through the jugular vein with the bipolar leads being positioned in the right ventricle. A Millar catheter containing two solid-state pressure transducers and a flowmeter was introduced through the carotid artery. One of the pressure transducers was positioned in the left ventricle and the other in the ascending aorta with the flowmeter. Electrodes forming lead II ECG were placed on the limbs of the animals. During the first part of the study, the parameters were measured in awake dogs. All parameters were measured during the control period, after infusion of the priming dose of 40 mg/kg ouabain and after each graded doses (0.15 mg/kg ouabain every 15 minutes) until ventricular arrhythmia was produced. Before each ouabain infusion, the right ventricle was paced 10 times (8 conditioning stimuli at 300 ms intervals, the 9^{th} stimulus at 150 ms interval, and the 10^{th} stimulus at 130 ms interval) to determine if non-paced ventricular ectopic activity (termed RVR for repetitive ventricular responses) occurred. The end-point of each experiment was taken when the dog developed the first ventricular premature depolarization either spontaneously or after cessation of pacing. The values of all parameters measured or computed were compared between the control (predose) record and the last record before the ventricular arrhythmia developed or was provoked. Twenty-four hours later, the dogs were anesthetized intravenously with thiopental sodium (15 mg/kg) and alpha chloralose (100 mg/kg), and the study was repeated. Upon completion, the dogs were euthanized before awakening.

The measurements from these studies formed the database to build the logistic regression model. The independent variable(s) in the model was/were the parameter(s) measured while the dependent variable was either control or arrhythmia condition. "Group", awake or anesthesia

was also included as a factor. Since this study is to investigate the feasibility of building a predictive model, a significance level of 0.10 was chosen as an inclusion criterion.

Swine Study: The swine were evaluated only under anesthesia. Thus, when constructing the model, "Group" was not included as a factor.

Statistical Methods: The purpose of this investigation is to construct a statistical model to predict the onset of cardiac arrhythmia when subjects are exposed to ouabain. Logistic regression was used because the out come variables were binary (Neter *et. al.*, 1983). For each of the parameters measured, a value of one was assigned as the dependent variable if the measurement was taken at the onset of arrhythmia and a value of zero was assigned if the measurement was recorded under control conditions. Logistic regression is given by equation 1 with y being the indicator response variable and x the corresponding cardiac parameter estimates.

$$E(Y) = \frac{\exp(\beta_0 + \beta_1 X)}{1 + \exp(\beta_0 + \beta_1 X)} \qquad \text{(Equation 1)}$$

To determine if more than one independent variable can be used to improve the predictive value of the model, a multiple logistic function is used. This function is obtained merely by adding an additional variable(s) to the exponent. From the variance/covariance matrix of the measured independent variables, it was observed that all of the variables found to be predictors were highly correlated. In this framework, a high correlation indicates

multicollinearity thus simple logistic functions are indicated. If the model is significant, (i.e. is a good predictor of onset of arrhythmia), a plot of the resulting will be curvilinear. However, if the model is not significant the fitted logistic function will be a straight line, indicating the independent variable under investigation is not a good predictor of onset of cardiac arrhythmia.

Results

Dog Study: In the construction of the model in dogs, the factor "Group" (awake vs. anesthetized) was not significant, thus both the awake and anesthetized subjects were combined. Five parameters, HR, PQ, QT, Paos and $dP/dt_{max.}$, were found to significantly predict the onset of arrhythmias. Figures 1(a,b) through 5 (a,b) illustrate these models. Figures 1a-5a include the awake and anesthetized model while Figure 1b-5b includes only the anesthetized model. There were no sets of two or more independent variables, which improved the predictive quality of the model.

Swine Study: PQ, QT and dP/dt_{max} were found to significantly predict the onset of arrhythmia by logistic regression (Figures 6, 7 and 8).

Discussion

The objective of this investigation was to determine if a mathematical model could be constructed to predict the onset of cardiac arrhythmia. Further, it was also important to determine if anesthesia had an effect on the model. This investigation indicates anesthesia has no

significant effect on the resulting model. The significance of this information is that the purpose for constructing the model was to predict the potential for a cardiac incident in man and certainly man will be awake when in an environment which exposes him to a potential gas. On the other hand, conducting the investigation under anesthesia was more humane for the dog and necessary for swine.

The parameters found to be significant predictors are obtained non-invasively. Thus if one wishes to use these models in practice, it is relatively easy to obtain these values in the mobile individual who may potentially be exposed to a gas. Logistic regression was used to develop these models because this function provides the user with an estimate of the probability of experiencing a cardiac arrhythmia. The conventional inferential statistics will only tell the user if control values of a parameter are significantly different from the values at the onset of arrhythmia. The only limitation of logistic regression is the event of all control values being contained in an interval such that there is no overlap with the interval containing the impending onset values. In this situation there is no single regression function, which will describe the data, instead there are an "infinite" number of functions which could describe the data.

In this study ouabain was used to provoke arrhythmias. Although digitalis is thought to provoke arrhythmias due to after depolarizations resulting from intracellular calcium overload, we do not know the precise mechanism(s) by which either ouabain or other cardiac sensitizers such as halons, CFCs and cocaine provoke ventricular arrhythmias. However all sensitizers probably favor the development of triggered activity (Opie, 1988; Boyden, 1996), particularly in the presence of increased catecholamine levels (Clark, *et.al.*, 1971) It is clear that

pharmacological responses to ouabain differ from those of halons. Ouabain, a cardiac glycoside, is a potent parasympathomimetic, a positive inotrope, and in normal animals a vasoconstrictor in higher doses. It is also a negative doromtrope. Halons, particularly at higher concentrations, are negative inotropes and are vasodilators, which decrease systemic vascular resistance (Aviado, 1974). Also at higher doses they share negative dromotropism with digitalis. Both compounds, by triggering premature depolarizations, may produce tachycardias by reentry due to slow conduction from the premature depolarizations. It is also known that some halons prolong QT, and that such prolongation in concert with autonomic effects should increase the tendency to produce torsades de pointes (Riley, 1988). Further, halons may be proarrhythmic by their potential to decrease oxygen delivery, to increase oxygen demand, therefore leading to possible depletion of phosphogen stores, the source of energy to sustain normal membrane physiology (Kagiyama, *et. al.*, 1982). It is important to emphasize that this model is applicable to detect a proarrhythmic effect induced by ouabain. There is no reason why the same methodology should not be applicable for other proarrhythmic interventions, however this study must be duplicated for halons— the target compounds of interest. Since both ouabain and halons may produce arrhythmias by a similar mechanism (i.e. triggered activity), it is not unreasonable to have used ouabain as a surrogate for halons.

It should be noticed that lengthening of QT in dogs and shortening of QT in pigs were predictors of arrhythmia. This can be explained by the different heart rate response in dogs compared to pigs, and with knowledge that QT is inversely related with heart rate in all species. Dogs responded to incremental doses of ouabain by decreases in heart rate, and this decrease in heart rate was clearly a more important determinant of QT than the ability of ouabain to

accelerate repolarization, (i.e. to shorten QT) (Cagin *et. al.*, 1974). A dog is a well-known parasympathotonic mammal, with an unusually high degree of heart rate variability. Thus, in pigs, the QT shortened consistent with the well-known effects of digitalis (ouabain), because there was not the dramatic decrease in heart rate, which would have prolonged QT. In any case dogs and swine were used because they represent large animals used commonly for physiological and toxicological evaluations, and because they have different features of cardiovascular physiology. That is, heart rate variability manifested as respiratory sinus arrhythmia is great in dogs and small in pigs, the pathways of ventricular activation differ greatly because of differing distributions of Purkinje fibers, and because QT adjusted for heart rate is much shorter in dogs than in pigs. Thus if the model provided good prediction in two species with widely different physiology, it would require less extrapolation for use in humans.

DISCLAIMER

This research was sponsored by the Navy CFC/Halon Replacement Program; NAVSEASYSCOM Code 03V2 and conducted at the Naval Medical Research Institute Detachment, (Toxicology). The experiments described herein were performed according to the principles set forth in the current edition of the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council. Opinions contained herein are those of the authors and are not to be construed as official or as reflecting the views of the Department of the Navy or the Naval Service at large. Mention of commercial products or services does not constitute endorsement of the Department of Navy or the Naval Service at large.

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Figure 1a: Comparison of probabilities for the onset of arrhythmia based on the heart rate (HR) of awake and anesthetized dogs.

Figure 2a: Comparison of probabilities for the onset of arrhythmia based on the PQ interval of awake and anesthetized dogs.

Figure 3a: Comparison of probabilities for the onset of arrhythmia based on the QT interval of awake and anesthetized dogs.

Figure 4a: Comparison of probabilities for the onset of arrhythmia based on the heart rate (HR) of awake and anesthetized dogs.

Figure 5a: Comparison of probabilities for the onset of arrhythmia based on the Paos of awake and ane sthetized dogs.

Figure 1b: Probabilities for the onset of arrhythmia based on the heart rate (HR) of anesthetized dogs.

Figure 2b: Probabilities for the onset of arrhythmia based on the PQ interval of anesthetized dogs.

Figure 3b: Propabilities for the onset of arrhythmia based on the QT interval of anesthetized dogs.

Figure 4b: Comparison of probabilities for the onset of arrhythmia based on the heart rate (HR) of anesthetized dogs.

Figure 5b: Probabilities for the onset of arrhythmia based on the Paos of awake and anesthetized dogs.

Figure 6: Probabilities for the onset of arrhythmia based on the PQ interval of anesthetized swine.

Figure 7: Probabilities for the onset of arrhythmia based on the Q T interval of anesthetized swine.

Figure 8: Probabilities for the onset of arrhythmia based on the PQ interval of anesthetized swine.

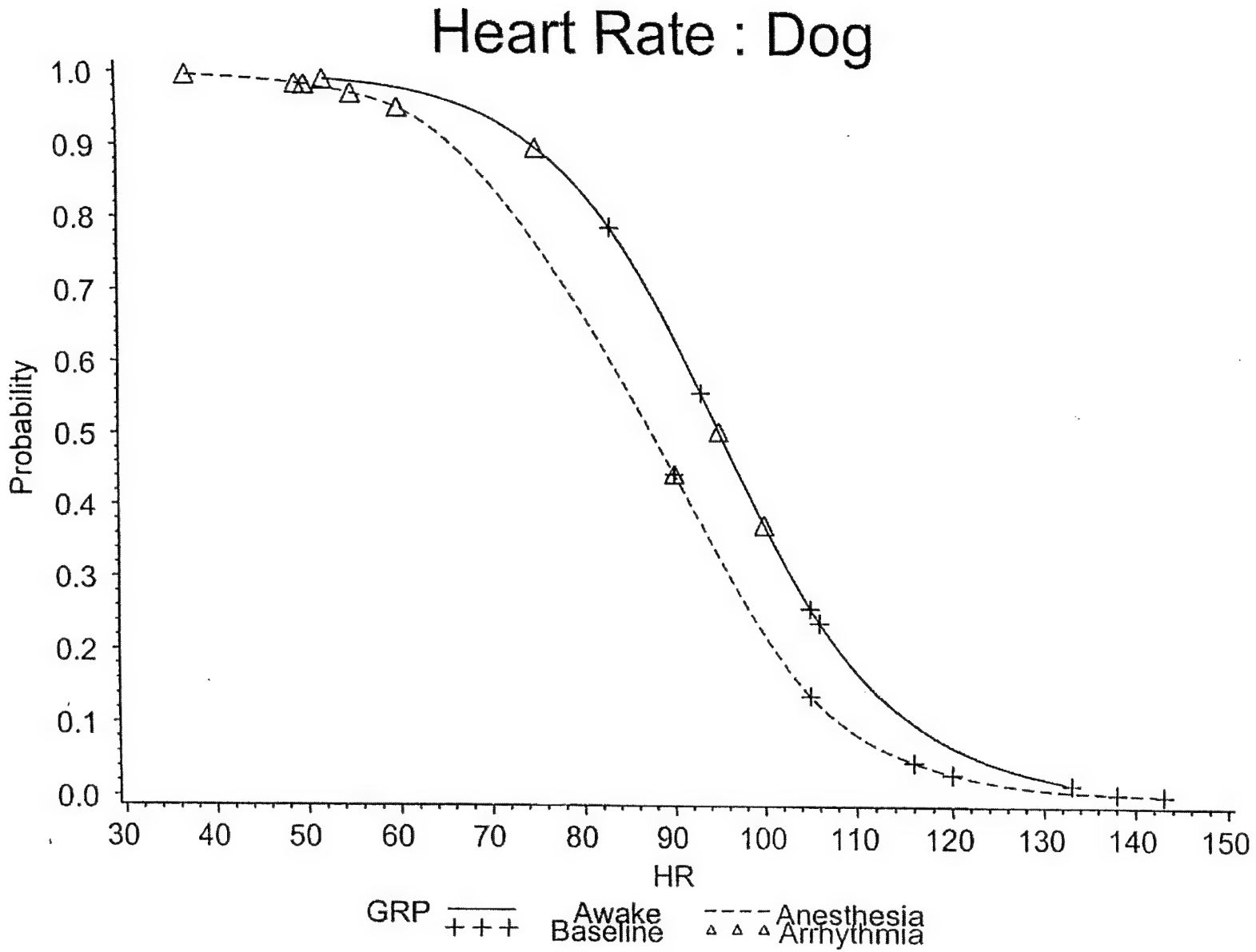

Heart Rate : Dog
Probability
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
30
40
50
60
70
80
90
100
110
120
130
140
150
HR
GRP
Awake
Baseline
Anesthesia
Arrhythmia

Figure 1a

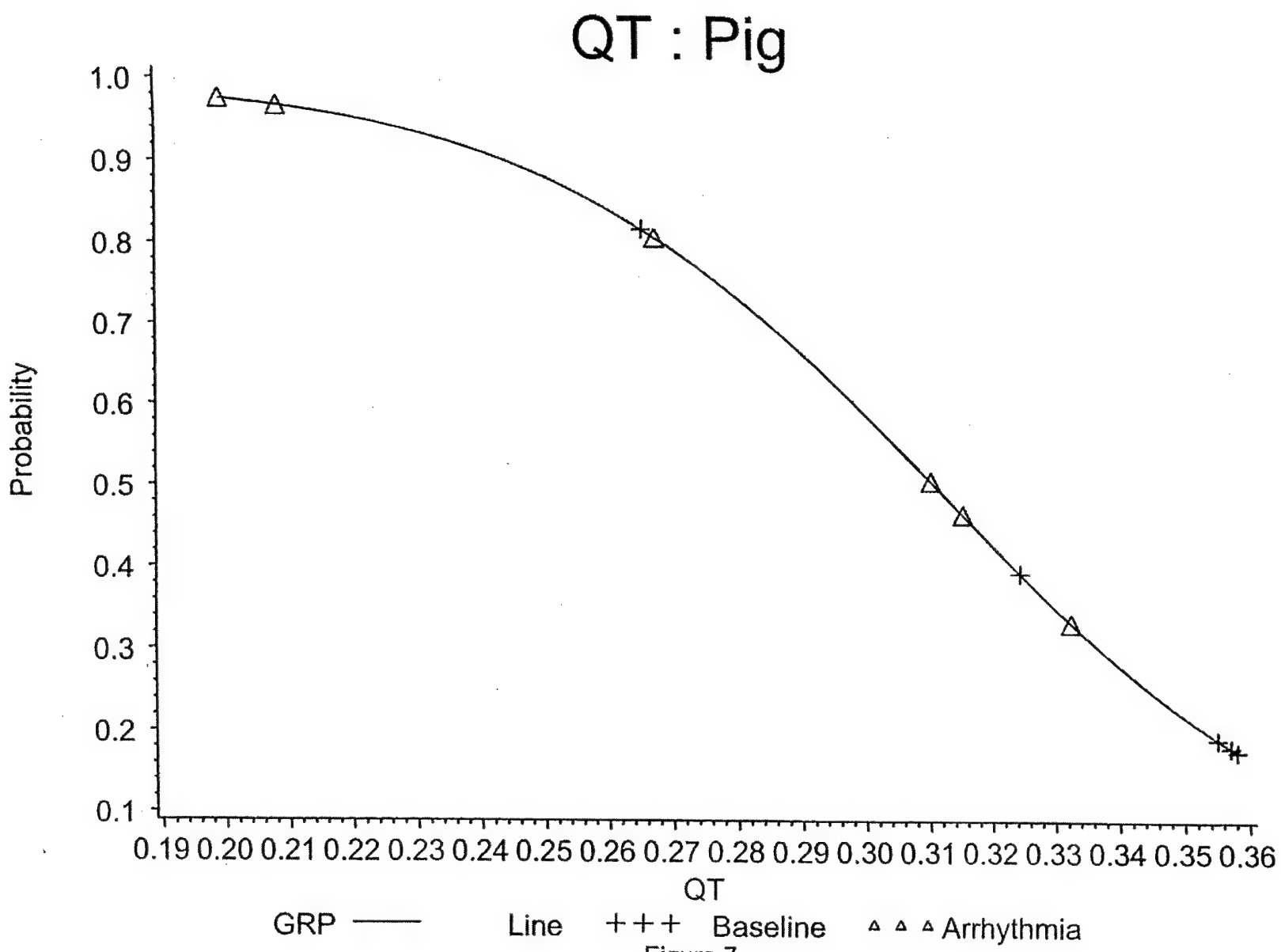

QT : Pig
Probability
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.19 0.20 0.21 0.22 0.23 0.24 0.25 0.26 0.27 0.28 0.29 0.30 0.31 0.32 0.33 0.34 0.35 0.36
QT
GRP —— Line +++ Baseline △ △ △ Arrhythmia

Figure 7

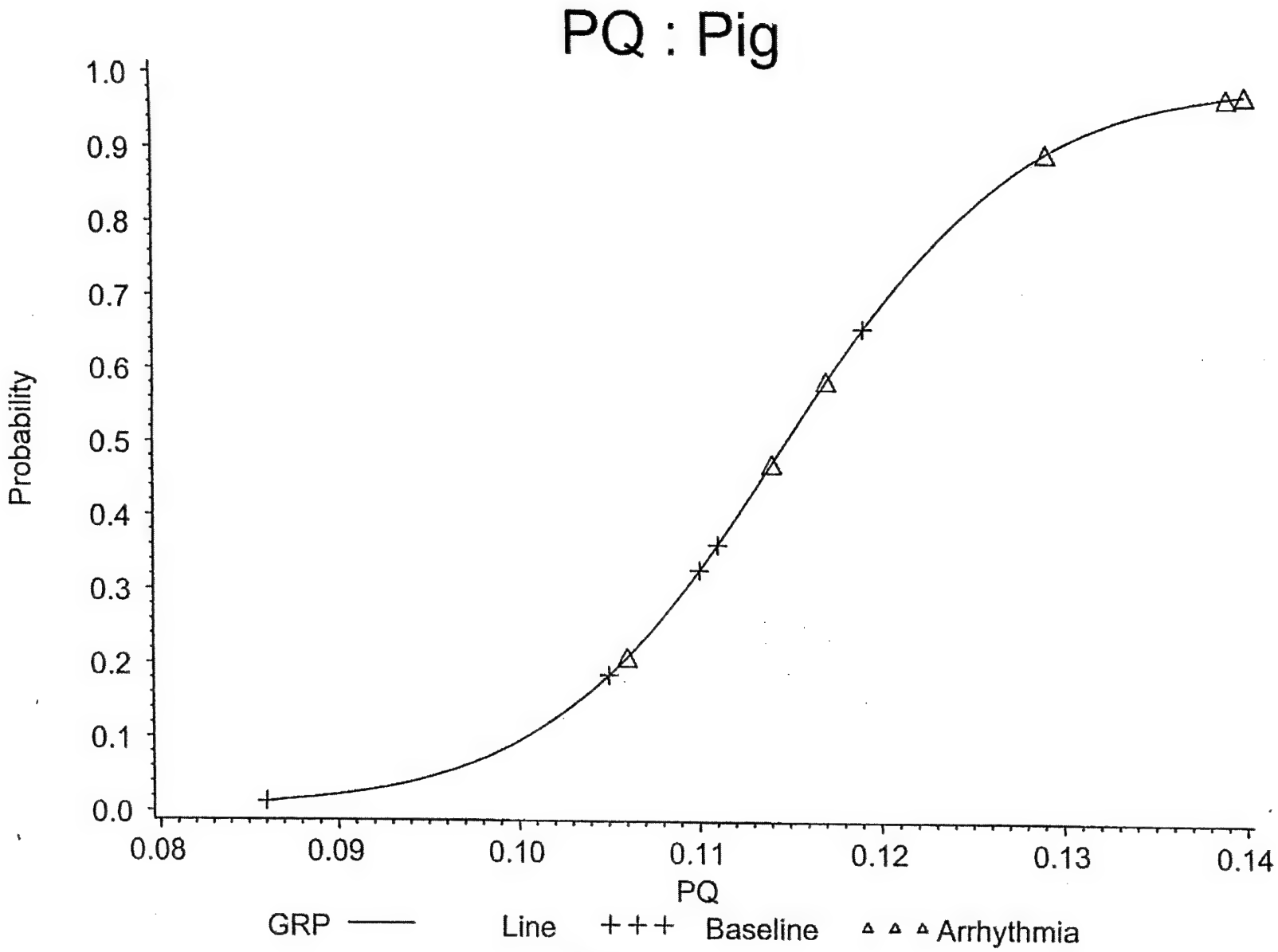
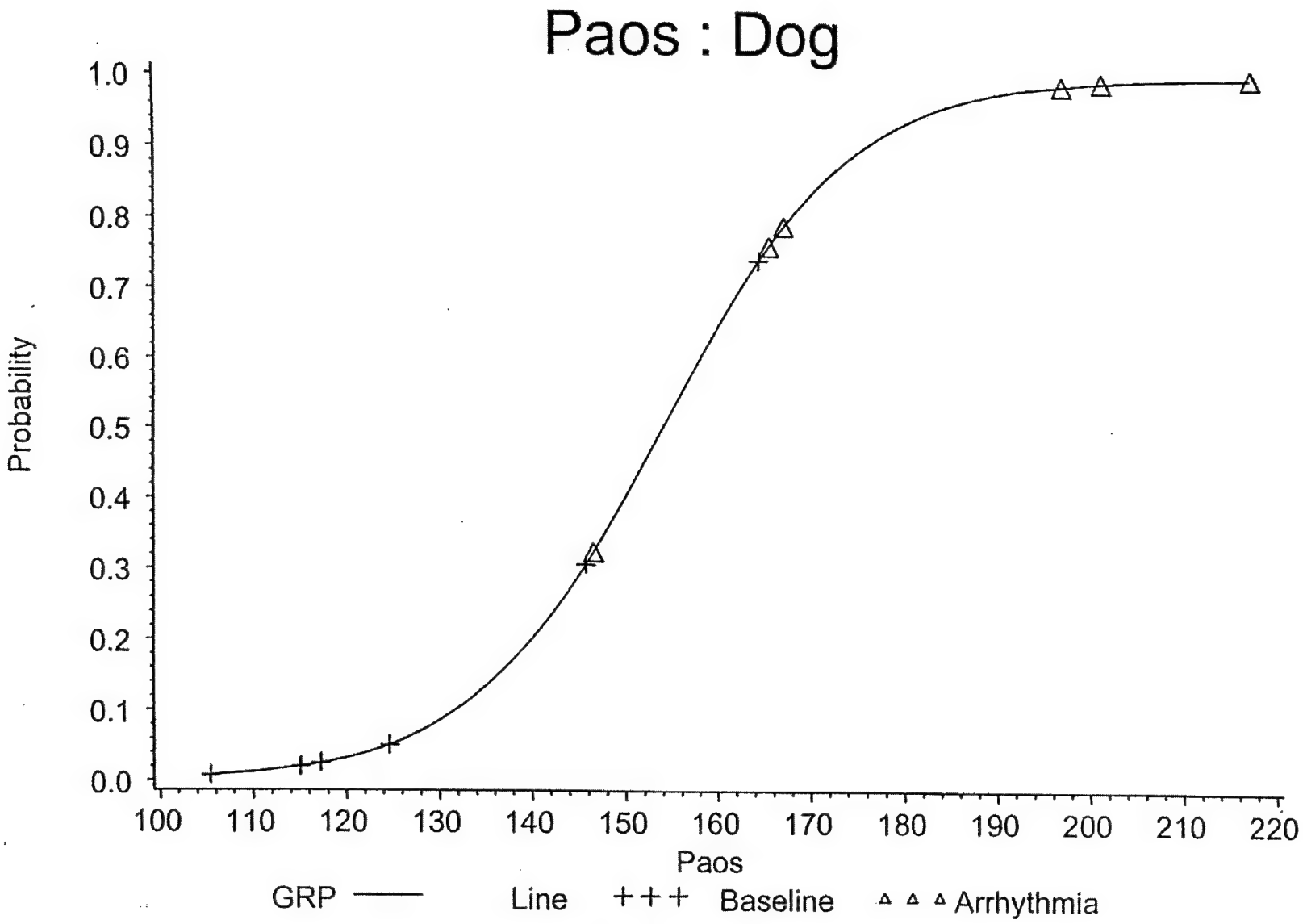
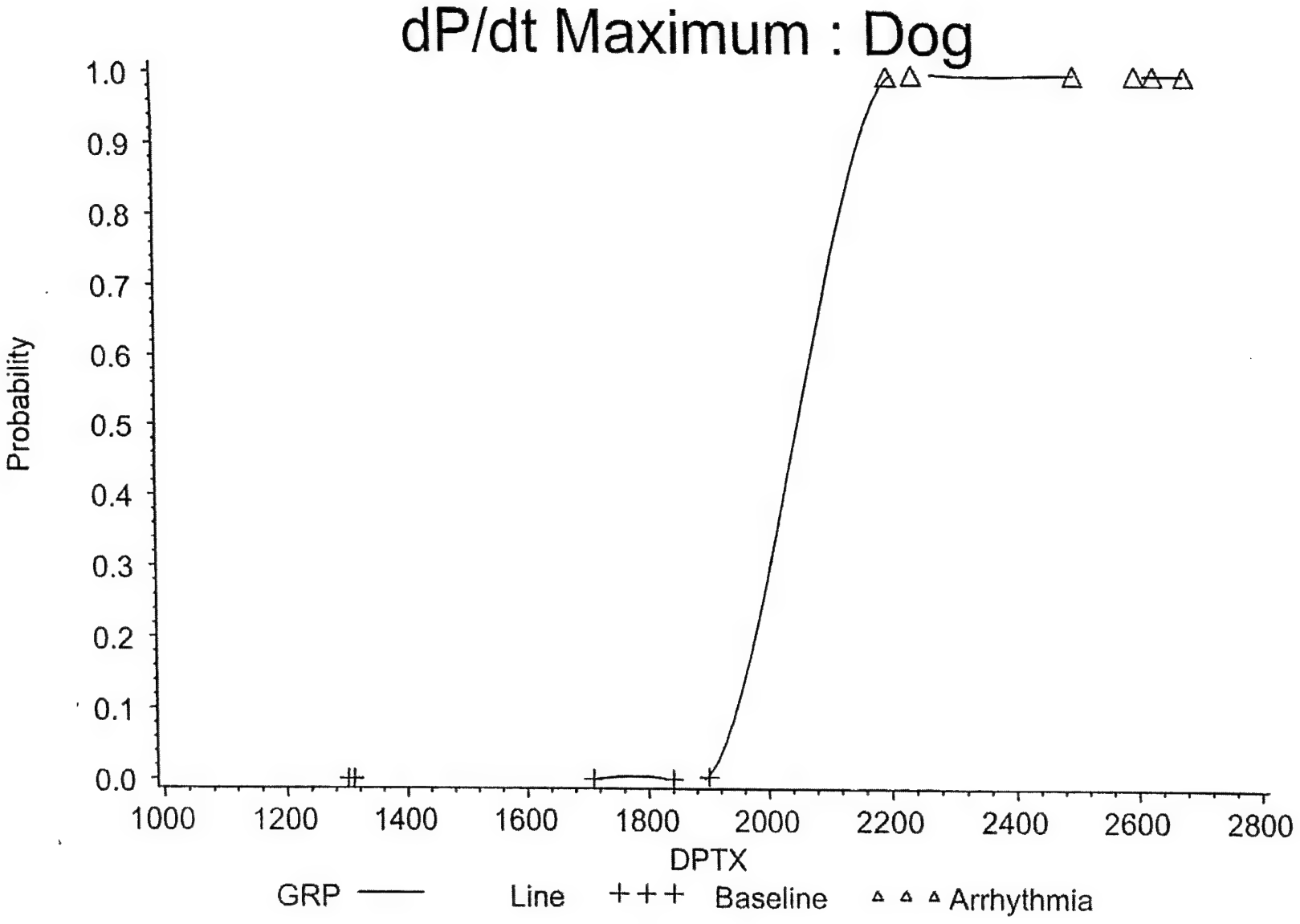

PQ : Pig
Probability
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.08
0.09
0.10
0.11
0.12
0.13
0.14
PQ
GRP
Line
Baseline
Arrhythmia

Figure 6



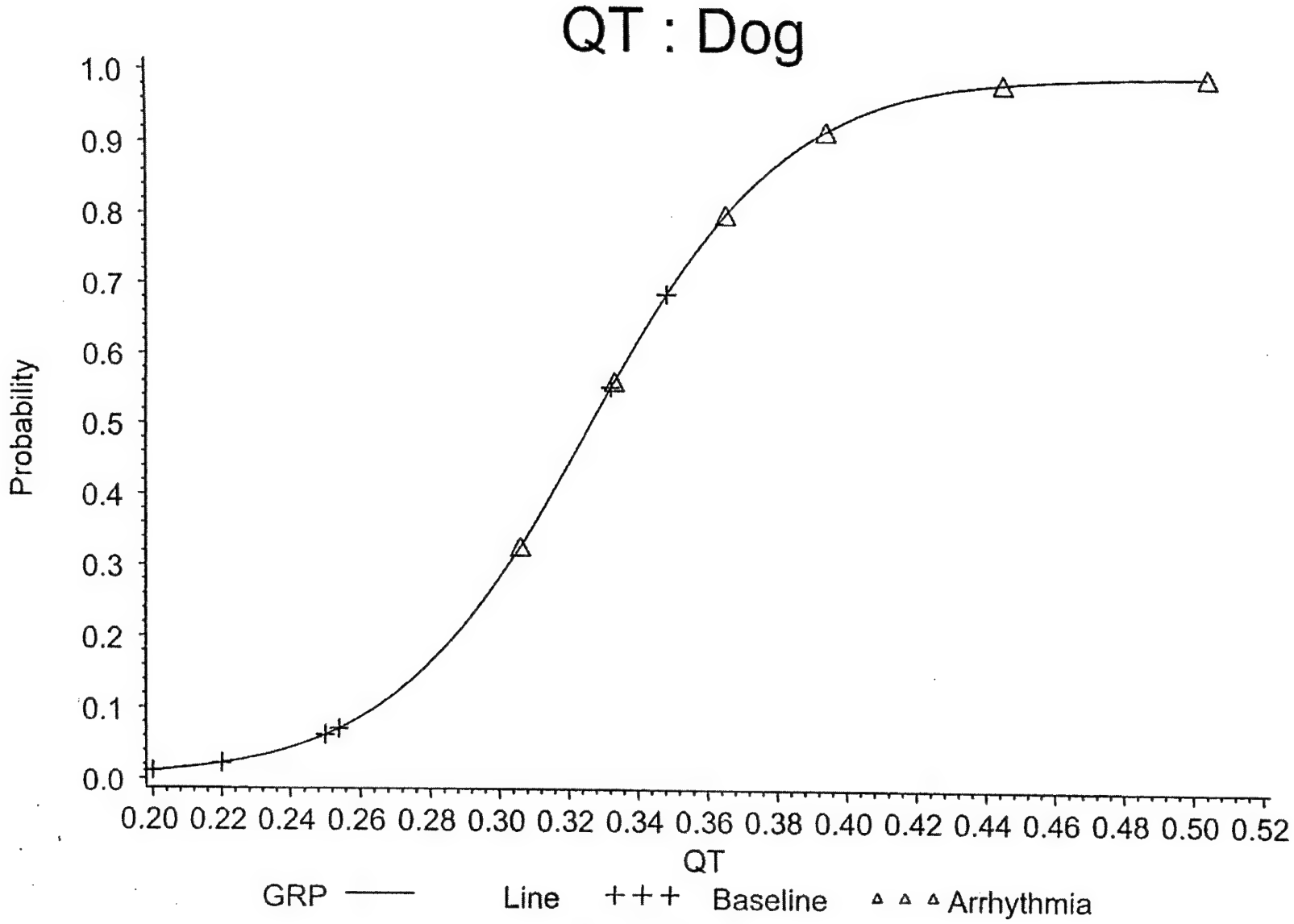
Anesthesia Only

Figure 5b



Anesthesia Only

Figure 4b


QT : Dog
Probability
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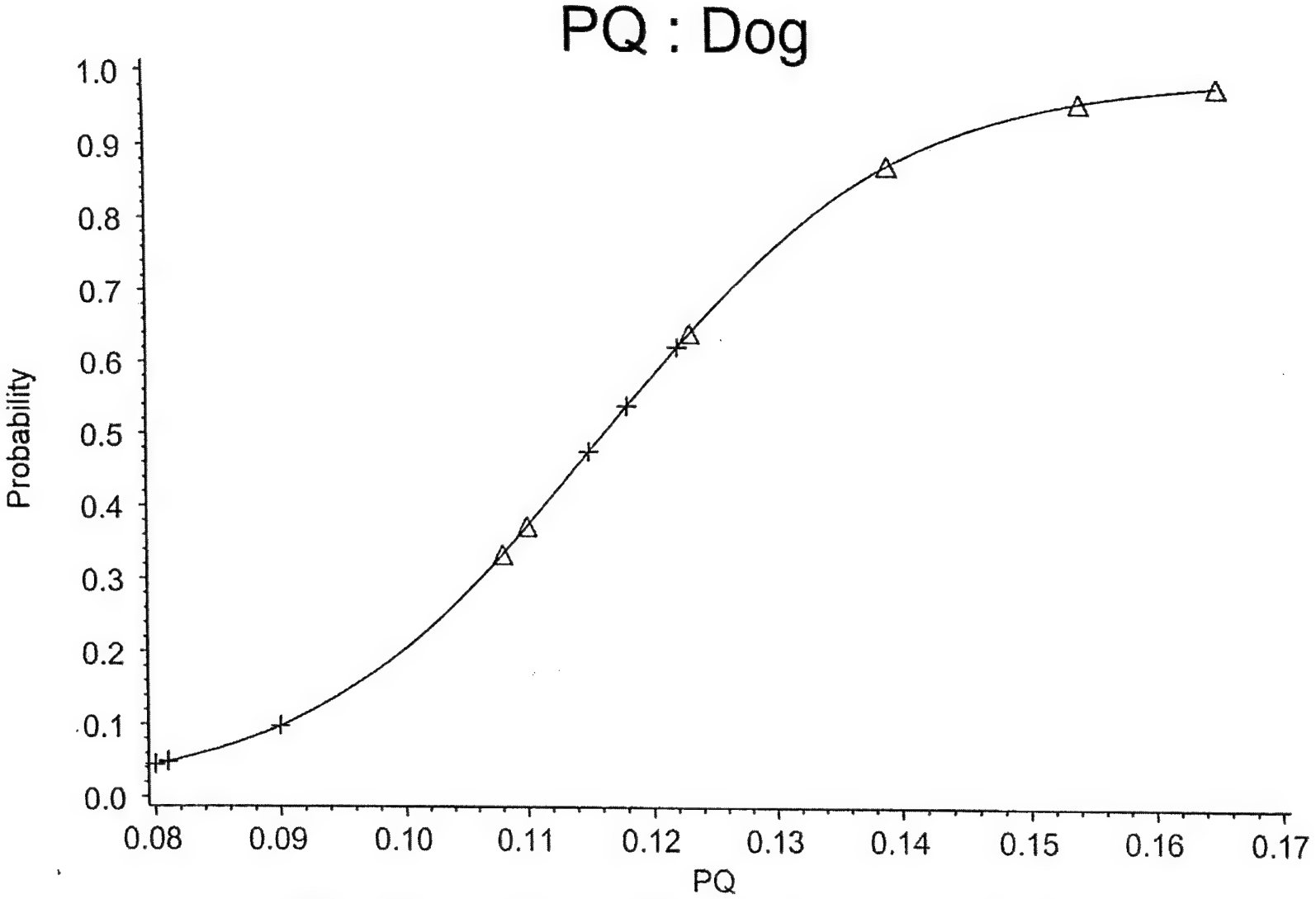
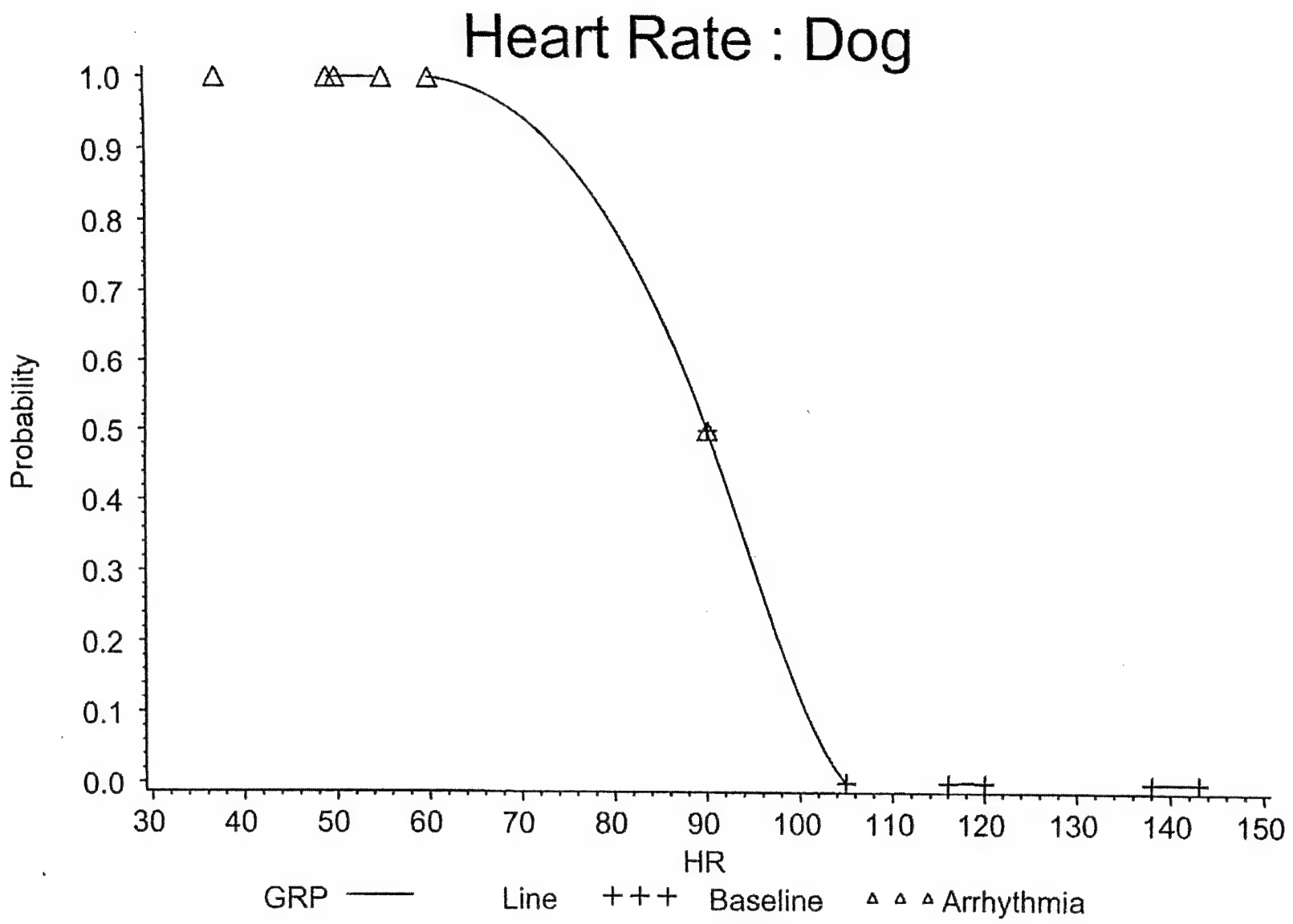
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.20 0.22 0.24 0.26 0.28 0.30 0.32 0.34 0.36 0.38 0.40 0.42 0.44 0.46 0.48 0.50 0.52
QT
GRP —— Line +++ Baseline △ △ △ Arrhythmia
Anesthesia Only

Figure 3b



Anesthesia Only

Figure 2b


Heart Rate : Dog
Probability
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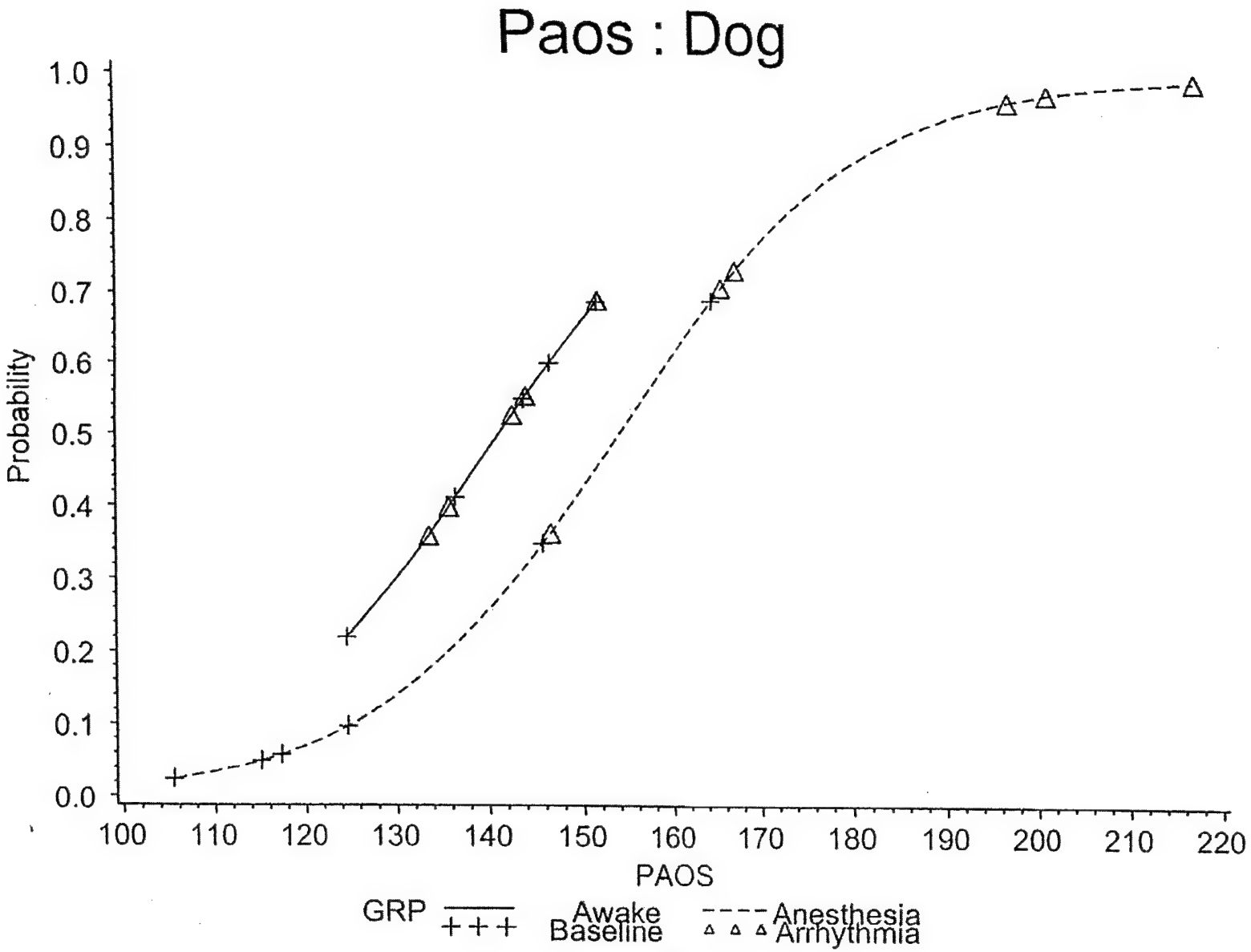
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
30
40
50
60
70
80
90
100
110
120
130
140
150
HR
GRP
Line
Baseline
Arrhythmia
Anesthesia Only

Figure 1b


Paos : Dog
Probability
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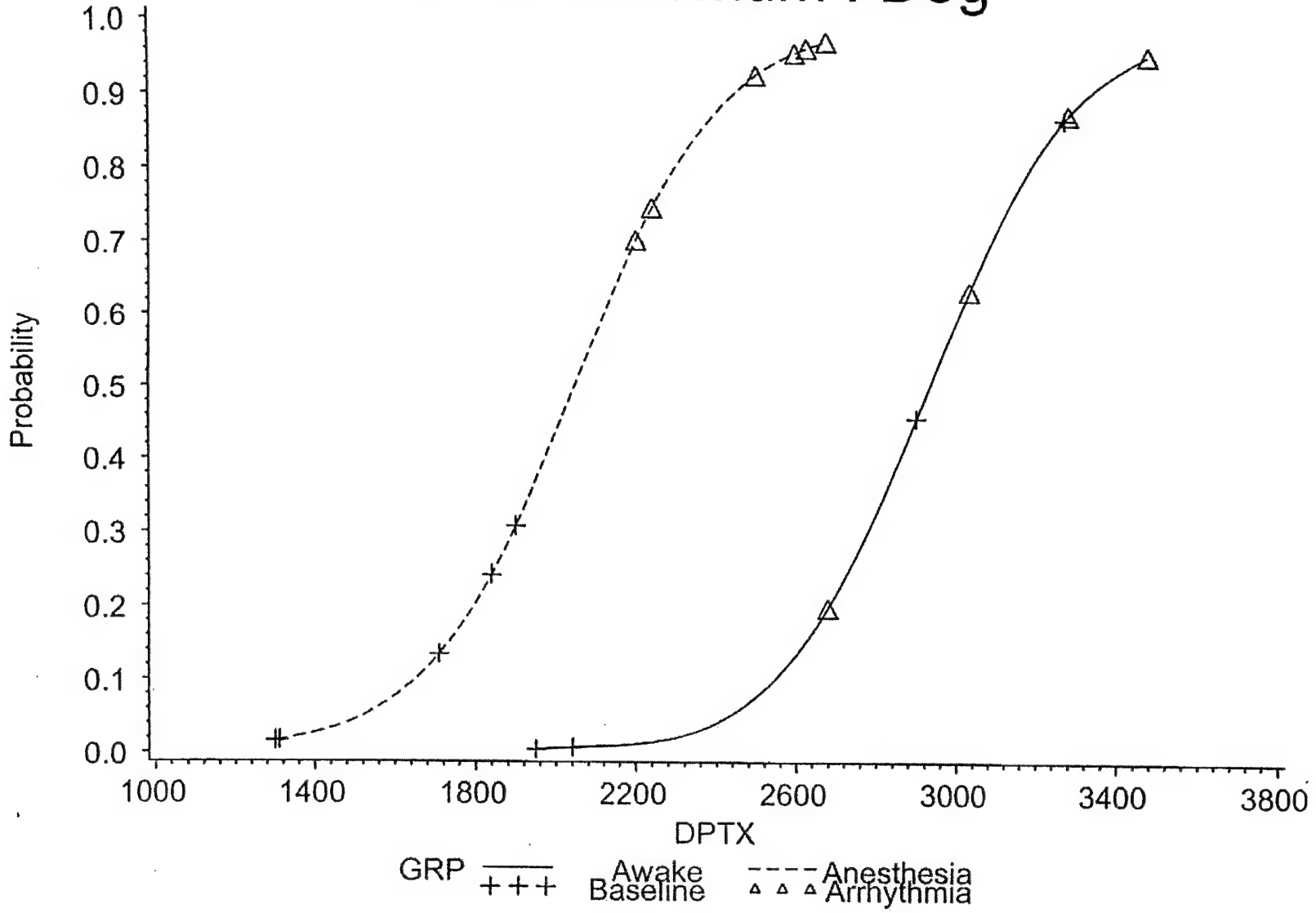
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
100
110
120
130
140
150
160
170
180
190
200
210
220
PAOS
GRP
Awake
Anesthesia
Baseline
Arrhythmia

Figure 5a


dP/dt Maximum : Dog
Probability
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0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1000
1400
1800
2200
2600
3000
3400
3800
DPTX
GRP
Awake
Anesthesia
Baseline
Arrhythmia

Figure 4a

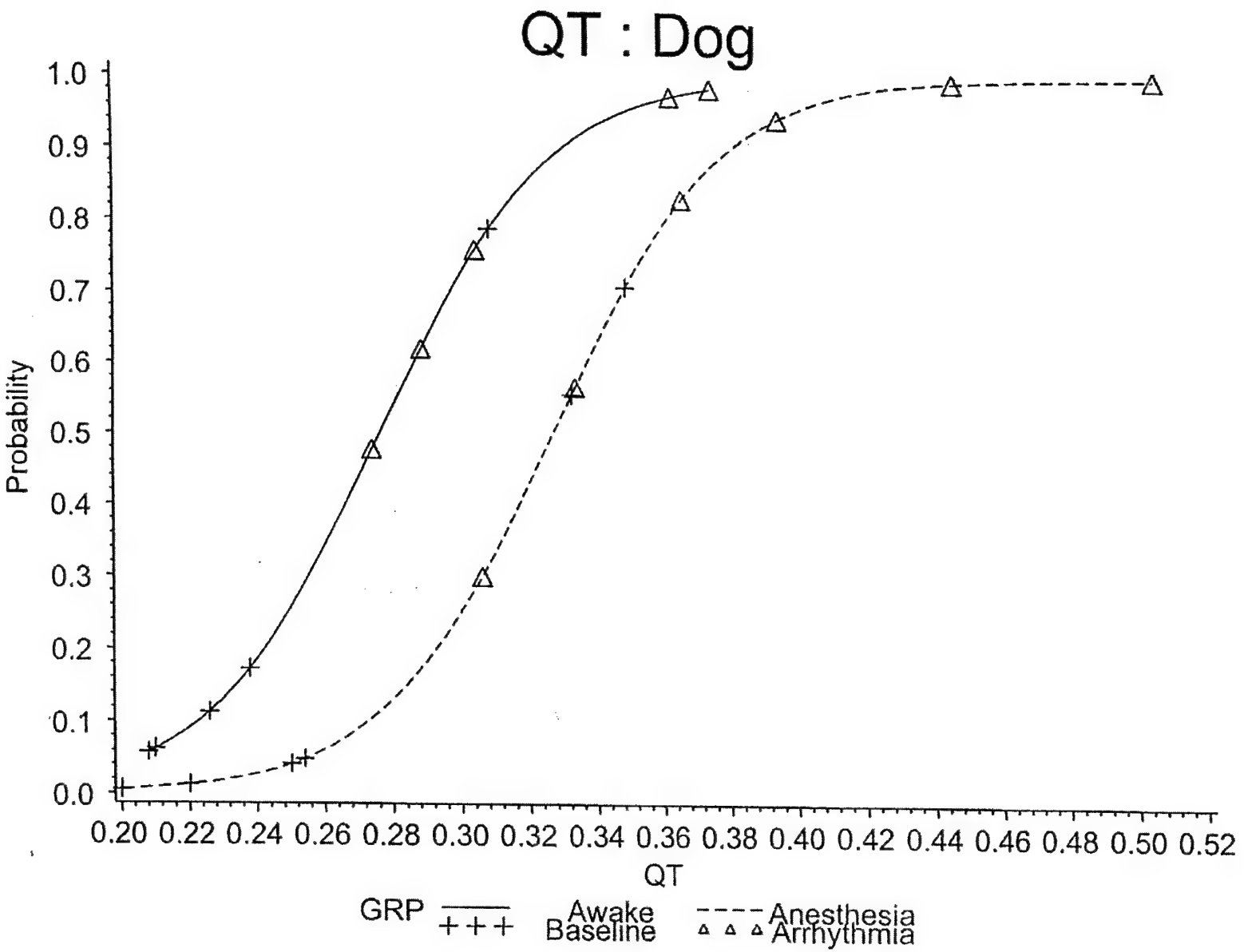
QT : Dog
Probability
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.20 0.22 0.24 0.26 0.28 0.30 0.32 0.34 0.36 0.38 0.40 0.42 0.44 0.46 0.48 0.50 0.52
QT
GRP
Awake
Baseline
Anesthesia
Arrhythmia

Figure 3a

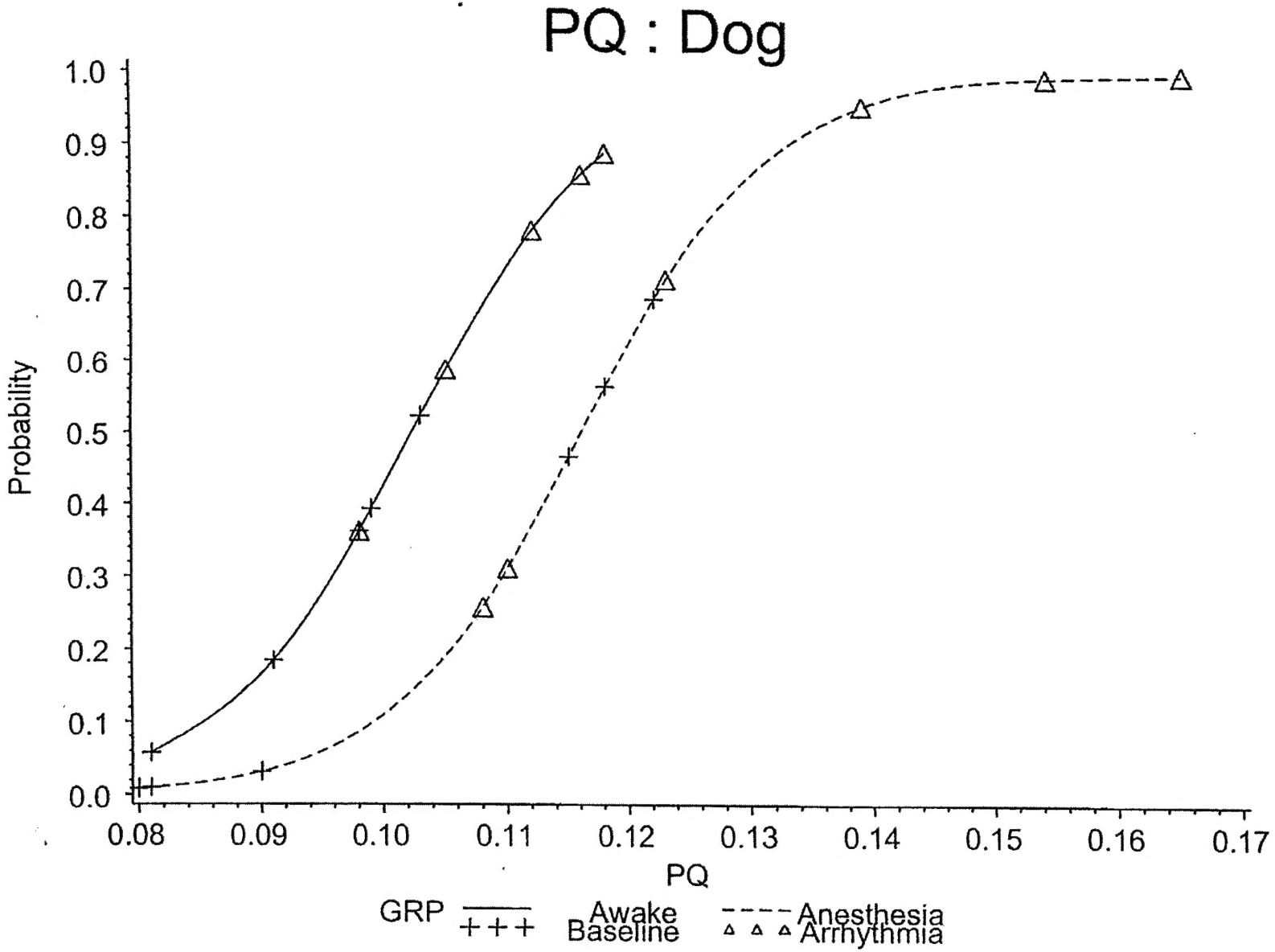
PQ : Dog
Probability
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.08
0.09
0.10
0.11
0.12
0.13
0.14
0.15
0.16
0.17
PQ
GRP
Awake
Anesthesia
Baseline
Arrhythmia

Figure 2a

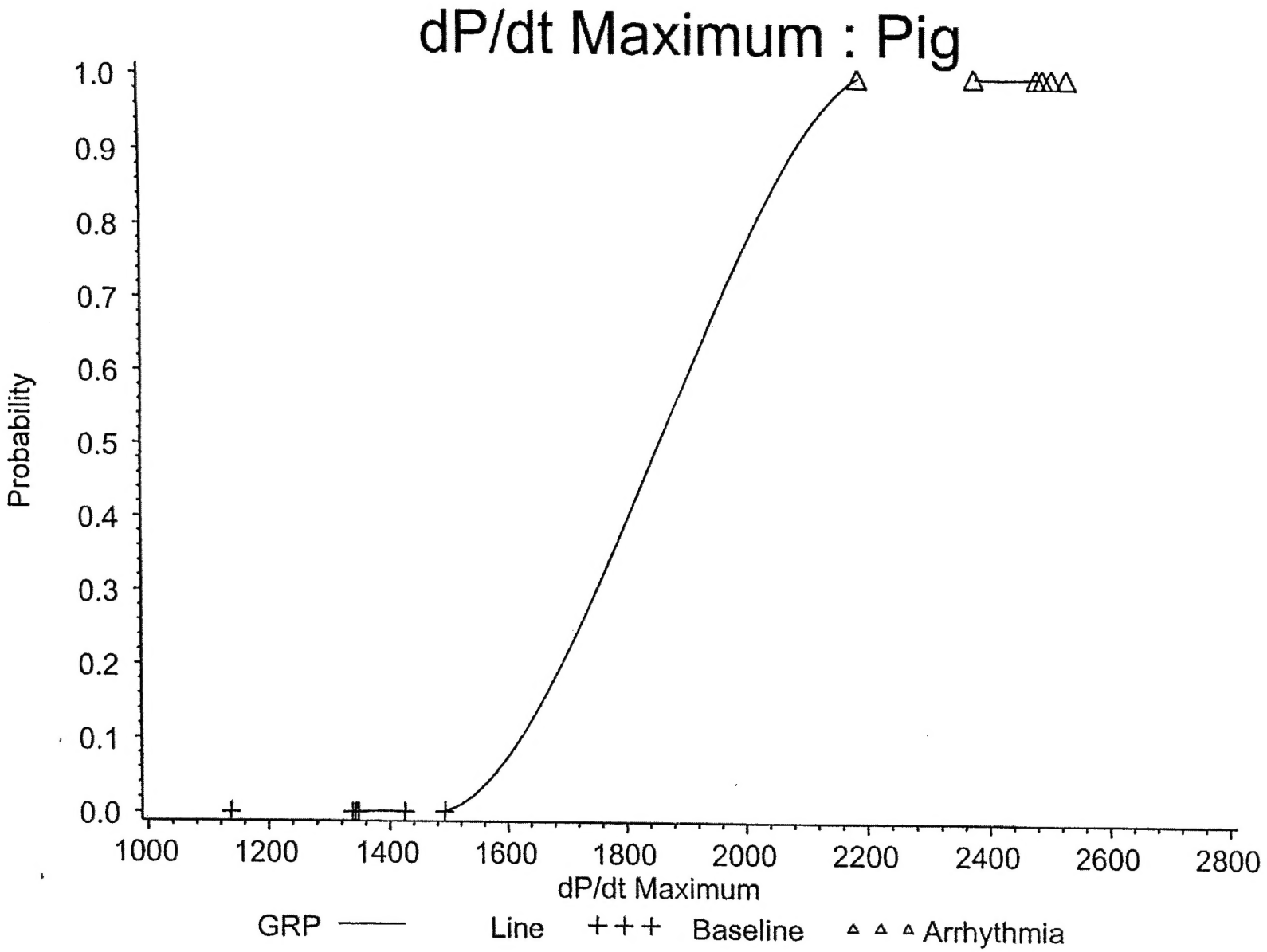
dP/dt Maximum : Pig
Probability
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
dP/dt Maximum
GRP
Line
Baseline
Arrhythmia

Figure 8

REPORT DOCUMENTATION PAGE

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1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	April 2003	April 2001 – April 2003

4. TITLE AND SUBTITLE
COMMENTARY AND SCIENTIFIC REVIEW OF STUDIES ON CARDIAC FUNCTION AND CARDIAC SENSITIZATION MODELS

5. FUNDING NUMBERS
TOXDET-03-06

6. AUTHOR(S)
Dr. William J. Brock, Dr. Edgar C. Kimmel, and CDR Warren W. Jederberg

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Naval Health Research Center Detachment Toxicology
NHRC/TD
2612 Fifth Street, Building 433
Area B
Wright-Patterson AFB, OH 45433-7903

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Naval Health Research Center Detachment Toxicology
NHRC/TD
2612 Fifth Street, Building 433
Area B
Wright-Patterson AFB, OH 45433-7903

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11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION AVAILABILITY STATEMENT
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12b. DISTRIBUTION CODE

13. **ABSTRACT** *(Maximum 200 words)* During the 1980's and into the 1990's, international agreements were developed that led to the phase out of the chlorofluorocarbons (CFC) because of the demonstrated effect these compounds have on depleting the protective ozone layer in the upper atmosphere. Because of the elimination of these compounds, the chemical industry began to introduce replacement compounds that have little or no effect on the ozone layer. These compounds, the hydrochlorofluorocarbons (HCFC) and hydrofluorocarbons (HFC), were introduced as replacements for the CFC in a variety of applications including refrigeration, aerosol propellants and fire extinguishing agents. In the last 15 years, several toxicological testing and research programs were initiated to examine the potential adverse health effects of these new alternatives. For the class of fluorocarbons, cardiac sensitization[4] remains a potentially serious health outcome of overexposure to these compounds. Although routine toxicological testing of these compounds normally includes an evaluation of this endpoint, it would be advantageous to the military use of these compounds if methods were developed that would allow prediction of cardiac sensitization before the onset of a fatal arrhythmia.

14. SUBJECT TERMS
chlorofluorocarbons (CFC); Halons; ozone depleting substances (ODS); Ouabain

15. NUMBER OF PAGES
100

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
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